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Research Articles

Hemodynamic Catheters: The Reprocessing, Cleanliness and in vitro Biofilm Formation by *Enterococcus faecium* in a Continuous Flow Model

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Abstract: Reprocessing single-use devices to cut costs is a common practice in hospitals around the world. In Brazil, there are few studies of reprocessing hemodynamic catheters and thus, this study aimed to evaluate the effectiveness of reprocessing hemodynamic catheters before and after biofilm formation in vitro using a continuous flow model. We used a sterility test and Scanning Electron Microscopy (SEM) to assess the presence of microorganisms, residue and integrity of a New (NC) and Reprocessed (RC) hemodynamic catheter, before and after in vitro biofilm formation by a clinical isolate of *Enterococcus faecium* (strain 155). NC was considered the negative control. The sterility test did not show the presence of microorganisms in either catheters used as a negative control (NC and RC). On the other hand, changes in integrity were observed by SEM in the RC, with a large number of microcracks and recesses, indicating that this would get worse after reprocessing. After biofilm formation and subsequent sterilization by ethylene oxide, both catheters were examined by SEM and RC showed a dense array of exopolysaccharide and substantial organic waste material, which was not evident in NC, showing changes in surface integrity. Ethylene oxide sterilization is very efficient in the sterilization process but the reprocessed catheters after biofilm formation by strain 155, showed marked surface changes, which increases the adhesion of organic matter and compromises the cleaning process in reprocessing. The results can be used as a parameter for hospitals and companies that reprocess catheters, to develop protocols for standardized and systematic surveillance in reusing materials recommended for single use to prevent infections.

Keywords: Single-use Devices, Catheter-Associated Infections, Sterilization, Residue, Scanning Electron Microscopy

Introduction

Reusing or not a manufactured product for medical and hospital has been a global question. There are those who support this practice aimed at reducing costs, since these products are quite costly for hospitals. However, there are many who question the lack of safety in the reprocessing of these products, where the cleaning and sterilization methods are not always effective in eliminating the presence of contaminants, which may cause serious problems for the patient, where a reprocessed and reused product is involved, such as the bacterial contamination established in biofilms and toxic waste.

Catheter-associated infections cause considerable morbidity and mortality in hospitalized patients. One of the difficulties associated with catheter infection is to establish the diagnosis. Evidence that a catheter is associated with an infection can be obtained when the catheter is removed and the distal end is subjected to microbiological evaluation. The isolation of the same microorganism from the tip of the catheter and the blood indicates a catheter-related infection (Elliott *et al*., 2000). In this regard, many methods are available for the culture of the catheter tip (Cleri *et al*., 1980; Collignon *el al.,* 1986; Maki *et al*., 1977; Raad *et al*., 1992). However, the gold standard is the culture of the catheter tip described by (Schinabeck and Ghannoum, 2003).

Bacteremia has been increasingly associated with the use of catheters from the continuous use of the gold standard for catheter tip culture. In this context, infection by vancomycin-resistant *Enterococcus* (VRE) arises as a significant problem among hospitalized patients (Edmond *et al*., 1996; Fridkin *et al*., 1999; Montecalvo *et al*., 1994; Newell *et al*., 1998). Furthermore, the rate of enterococcal bacteremia associated with central venous catheters increased progressively during the past two decades, (Gray *et al*., 1994; Malone *et al*., 1986; Patterson *et al*., 1995; Shlaes *et al*., 1981).

Thus, the use of *Enterococcus faecium* in vitro biofilm experiments is of high importance, since bacteremia caused by *E. faecium* associated with catheters has caused great concern in recent years, not only because of the difficulty of controlling the microorganism but also because of its virulence.

Gray *et al.* (1994) reported in their study that 35% of catheter-related infections of the bloodstream were caused by enterococci. Moreover, several studies have reported VRE episodes related to catheter use (Edmond *et al*., 1996; Lai, 1996; Moellering *et al*., 1999; Sandoe *et al*., 2002).

The most important factor in the pathogenesis of infections associated with foreign objects is the ability of bacteria to form biofilms on different surfaces of such devices. Biofilm is defined as a layered community of sessile microorganisms in an extracellular polymeric matrix produced by the microorganism, formed in an organized process (Donlan and Costerton, 2002). This process starts with the rapid and primary adhesion of bacterial cells onto the substrate surface followed by extracellular matrix coating. Next, the bacteria proliferate and accumulate, forming multilayered cell aggregates incorporated into the extracellular matrix (Speziale *et al*., 2008).

Studies have focused on standardization and researchers working in the area of reusing single-use materials have published the validation of cleaning products for repeated reuse. The cleaning process, whether manual or automated, has as main objective the elimination of possible waste and/or protein aggregates that could generate sources of contamination, or even facilitate the formation of new biofilm in the lumen of the reprocessed catheter (Alfa and Nemes, 2003).

Simulating a process is one of the most effective ways to conduct an evaluation, as well as finding the best way to achieve low levels of contamination that do not cause reactions or infection in the patient. Accordingly, this study aimed to evaluate possible changes on the inner surface of reprocessed and sterilized hemodynamic catheters and formation of biofilm by the clinical isolate *E. faecium* 155 VRE in an in vitro continuous flow model before and after reprocessing and sterilization using ethylene oxide.

Material and Methods

Bacterial Strain

The bacterial strain of biofilm formation used in the experiments was the clinical isolate *E. faecium* 155 VRE originating from cultures that were discarded as biological waste at the Laboratory of Microbiology of University Hospital and Clinic Center, Londrina, Brazil. These wastes were from tests performed in the routine care of hospitalized patients. Samples did not have any information that could identify the patient. The clinical isolate was kept in 40% glycerol at -4°C.

Culture Medium and Inoculum

The culture medium used in the experiments with in vitro of continuous flow and microbiological testing was Brain Heart Infusion (BHI). The inoculum of *E. faecium*, 155, was prepared from a liquid culture of cells in exponential growth phase. The initial inoculum for the continuous flow was adjusted to 10^4 (colony forming units) CFU/mL in a spectrophotometer at 600 nm.

Characteristics of the Catheter

The present study was carried using hemodynamic catheters (Performa® 5f, VERT). The unused catheters were obtained straight from the supplier and those reused five times were kindly donated to us by University Hospital and Clinic Center, Londrina, Brazil.

The new catheters and reprocessed catheters were evaluated by Scanning Electron Microscopy (SEM) in two steps, using three random replicas each. In the first (controls), we evaluated the New Catheter (NC) and the reprocessed catheter reused five times (RC). In the second (assay), samples of the NC and RC were subjected to biofilm formation by continuous flow, nominees were NCB and RCB. Afterwards, subsamples were subjected to direct evaluation by SEM and other subsamples were subjected to reprocessing (NCBR and RCBR). Subsamples of the NCBR and RCBR were subjected to sterility assay and other subsamples were evaluated by SEM (Fig. 1).

In Vitro Continuous Flow Model

For biofilm formation, a continuous flow system was constructed by joining the catheters to an Erlenmeyer flask (feed flask) containing BHI liquid medium with inoculum of *E. faecium*, strain 155 VRE, adjusted to 10^4 CFU/mL and another Erlenmeyer flask (receiver flask) (Fig. 2).

For in vitro biofilm formation, the flow was set at 1 mL/min using a pressure pump. Afterwards, the inoculum was carried through each catheter for 15 h and the whole system was maintained at 37°C.The guidelines of *Agência Nacional de Vigilância Sanitária* - ANVISA (Brasil, 2012) were used for reprocessing and sterilization of catheters.

Processing Protocol

Following the continuous flow experiments, the samples for reprocessing were placed, for ten minutes, in a warmed container filled with 4 mL of enzymatic detergent (Max Zyme®). Afterwards, the samples were dried and forwarded to a private company to be reprocessed and sterilized by ethylene oxide.

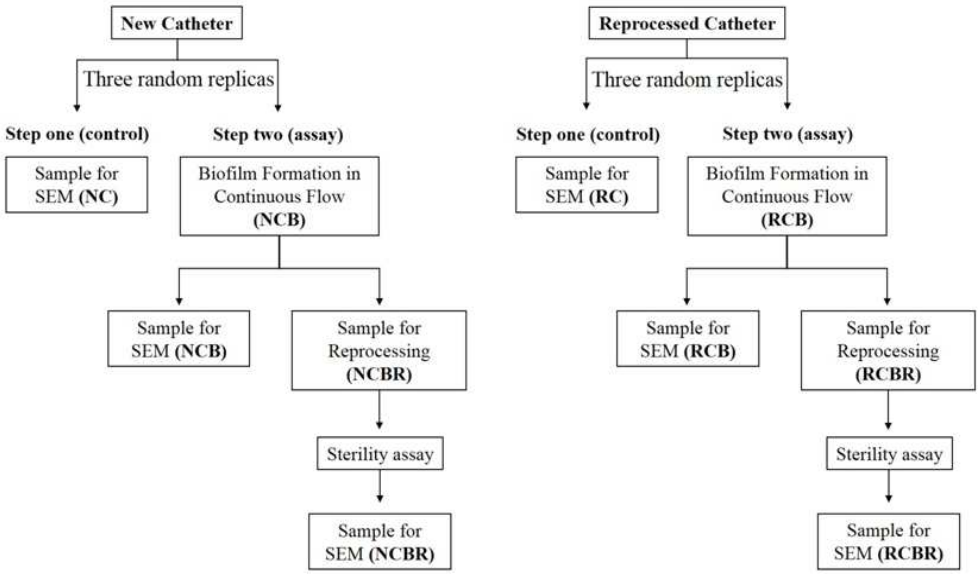

Fig. 1. Organizational chart of the assay

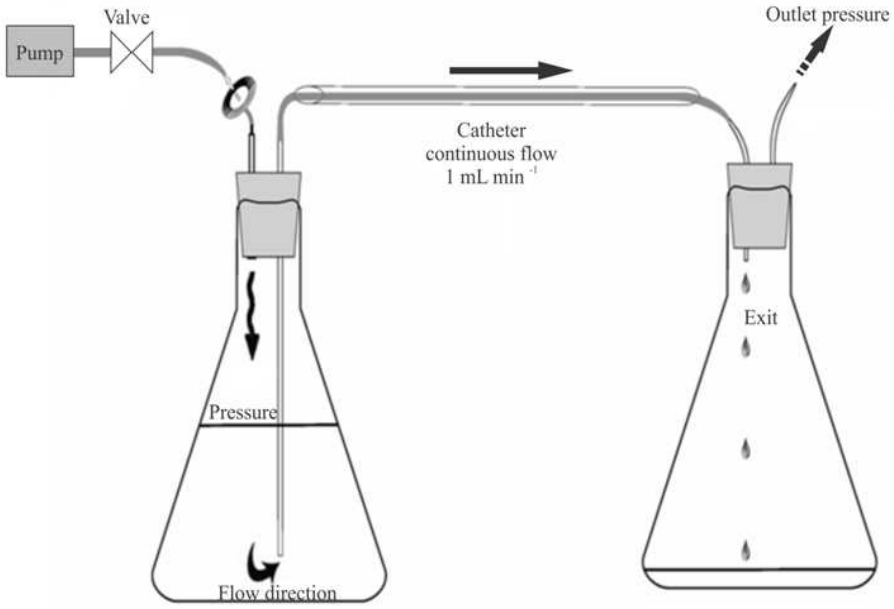

Fig. 2. Schematic depiction of the continuous flow model, including pressure pump, feeding and waste flasks and angioplasty catheter

Microbiological Evaluation

Samples were aseptically divided into 5 cm pieces, where 3 random replicas were used for microbiological evaluation and further incubated in BHI liquid medium for 24 h at 37°C. Next, microbial growth was determined according to medium turbidity.

Scanning Electron Microscopy

The samples for Scanning Electron Microscopy (SEM) evaluation were fixed by immersion in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7, for 12 h and post-fixed in 1% OsO_4 for one hour. The fixed material was then dehydrated in an ethanol gradient (70, 80, 90 and 100 GL). Next, the samples were critical point dried with CO_2 (BALTEC CPD 030 Critical Point Dryer), fixed on a support, coated with gold (BALTEC SDC 050 Sputter Coater) and examined by SEM (FEI Quanta 200).

Results

The strain 155 VRE, formed a high biofilm amount on the surface of the angioplasty catheters tested. The sterility testing of NCBR and RCBR samples, which were subjected to the in vitro biofilm formation test and then reprocessed and sterilized by ethylene oxide, showed no microbial growth after incubation.

SEM demonstrated that the NC surface was free of residue (Fig. 3A). In the higher magnification was observed a intact surface (Fig. 3B). On the other hand, was found great microcracks, roughness and residue inside the lumen of RC (catheter reused five times) in the small and in the higher magnification (Fig. 3C and 3D).

After biofilm formation by *E. faecium* VRE 155 in a continuous flow model, SEM analysis revealed an exopolysaccharide matrix on NCB (Fig. 4A and 4B). However, a large amount of this material was observed in the RCB (Fig. 4C and 4D). This suggests that the numerous microcracks and roughness on the RCB foster the adherence and establishment of VRE 155.

After reprocessing, was observed that NCBR was almost completely free of residue (Fig. 5A). However, already had small alterations in the lumen (Fig. 5B). On the other hand, in the RCBR was observed high amount of residue, roughness, microcracks and a large deformation (Fig. 5C and 5D). These observed changes by electron microscopy indicate that repeated processing render useless of the catheter.

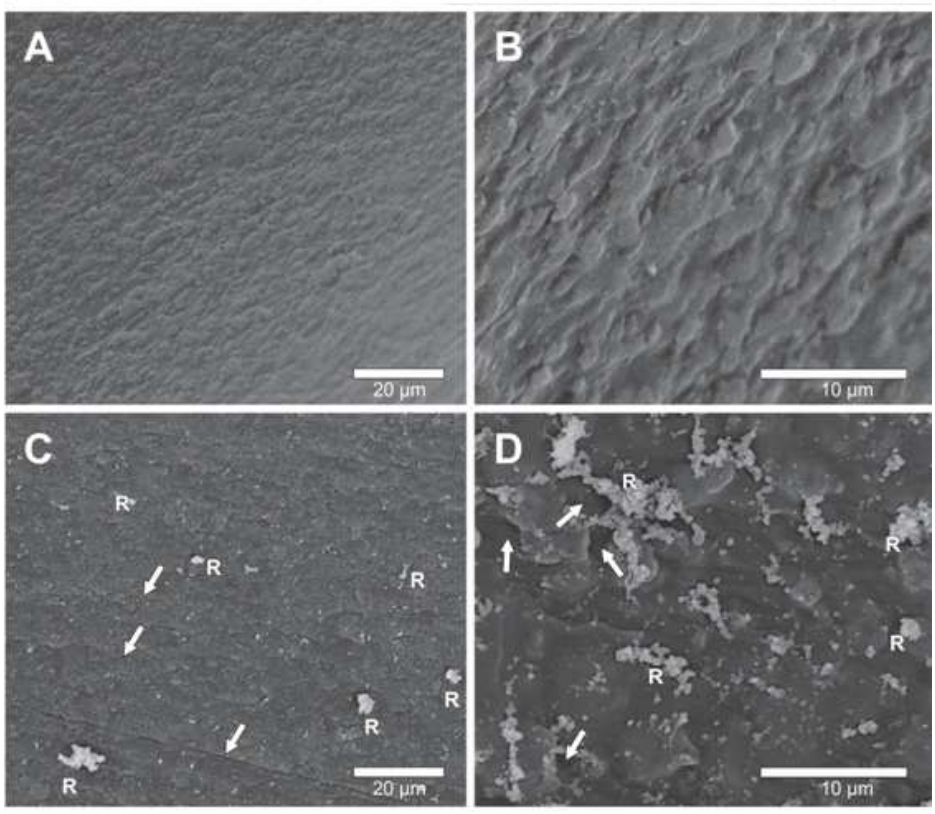

Fig. 3. Scanning electron microscopy of angioplasty catheter. A: new catheter (NC); B: higher magnification of NC; C: reprocessed catheter reused five times (RC); D: higher magnification RC. Arrow: microcracks; R: residue

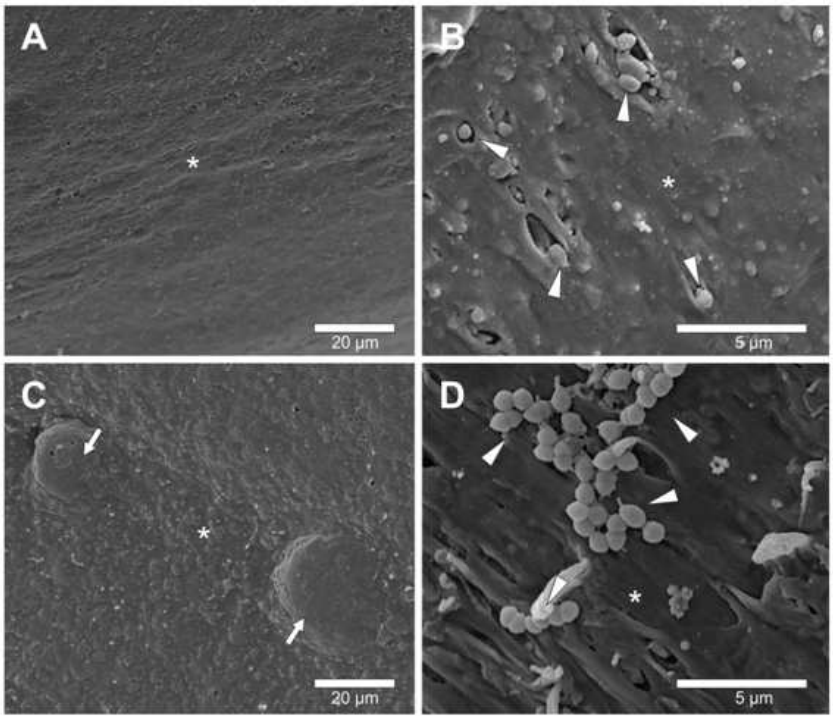

Fig. 4. Scanning electron microscopy of angioplasty catheter. A: new catheter subjected to biofilm formation by continuous flow (NCB); B: higher magnification of NCB; C: reprocessed catheter reused five times subjected to biofilm formation by continuous flow (RCB); D: higher magnification of RCB. Asterisk: biofilm layer; Arrow: mature biofilm; Arrowhead: *E. faecium* strain 155 VRE

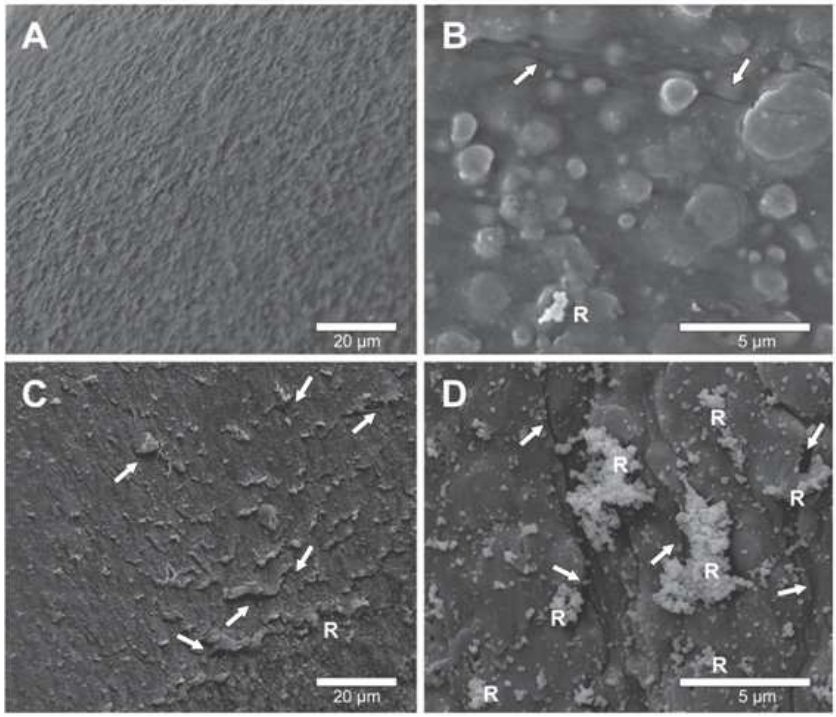

Fig. 5. Scanning electron microscopy of angioplasty catheter. A: new catheter subjected to biofilm formation by continuous flow and reprocessed (NCBR); B: higher magnification of NCBR; C: reprocessed catheter reused five times subjected to biofilm formation by continuous flow and reprocessed (RCBR); D: higher magnification of RCBR; Arrow: microcracks; R: residue

Discussion

Catheterism is one of the best discoveries in recent decades for diagnosis and therapy fields. The clinical procedure and manufacturer stipulate that a catheter is for a single use. Catheters are expensive and to decrease costs, many hospitals choose to reuse catheters (Pinto and Graziano, 2000). The risk of reprocessing this kind of accessory could contribute to increased risk of infection of the patient even though the catheter is cleaned up and sterilized, guaranteeing the absence of microorganisms or toxic agents and, especially, the retention of the functional characteristics of such products for reuse (Silva, 2005).

The presence of residue on reused catheters permits the adherence of bacteria as shown in the present study. The residue could contain exogenous pyrogens produced by bacterial metabolism. The large an amount of residue found in reprocessed catheters (before and after the continuous flow in biofilm formation test) could explain the importance of single use of the accessory.

Many authors have reported the difficulty of cleaning devices, in assessing the problems on the surface of reused catheters (Krause *et al*., 2000; Penna and Ferraz, 2001). By microscopy, it was possible to observe the presence of many cracks and roughness, which promote the adhesion of organic matter, decreasing the efficiency of cleaning processes.

In this study, we found that the catheters after reprocessing showed the presence of substantial residue, which leads us to conclude that reprocessing procedures are relatively inefficient in cleaning the catheter lumen. However, a study comparing infection risk in patients undergoing cardiac catheterization with disposable versus reprocessed catheters found that no patient developed infection (Frank *et al*., 1988). Similarly, in a survey of 12 large medical centers, reuse of pacing catheters was common and was not associated with post-procedure infections (O'Donoghue and Platia, 1988).

According to Brazil's regulatory agency (Brasil, 2012), the processing of catheters demands a validation protocol granted by the Health Bureau. However, even though the reuse of medical devises is common in developing countries, the procedures do not assure catheter integrity, as demonstrated in this study.

For many devices that are commonly reused, clear protocols for reprocessing and sterilization exist. However, for many single-use devices, such protocols do not exist and institutions that reprocess such devices may not even have their own internal protocols.

According to Hussain *et al*. (2012) if a product can be economically reprocessed with validated protocols and deemed to be functional, there is no reason to discard it after one use. If working models of safe sterilization and reprocessing can be achieved, it will be of use to both the patient and the environment. On the other hand, Shuman and Chenoweth (2012) believe that there may be cost savings, but that the degree to which savings are offset by adverse events is uncertain. More research is needed to help answer these important questions. In addition, there are many potential legal and ethical issues related to the reuse of single-use devices, again stemming from the lack of standards and data regarding adverse events.

According to Pantos *et al*. (2013), the reuse of Percutaneous Coronary Intervention (PCI) catheters is not generally recommended, since there are contradictory conclusions as far as patient safety is concerned.

Between December 1999 and July 2001, Amarante *et al*., (2008) conducted a survey using a questionnaire that was sent to 240 institutions affiliated with the Brazilian Society of Hemodynamics and Interventional Cardiology. Of the 119 institutions that participated in the survey, 97% stated that they reprocessed single-use utensils. Of these, 20% reported reuse less than five times before disposal, 38% reuse ranging from 5 to 10 times, 15% reuse from 11 to 20 times and 11% over twenty-times reuse. In that same survey, 13% reported having no control over reuse.

The safety conditions presented by the reuse of single-use item still expose the patient to infection risk, as suggested by Greene (2004). The concerns of the Association for the Advancement of Medical Instrumentation (AAMI) with regard to reuse include infection, pyrogens, toxic residue, functional reliability and physical integrity.

Conclusion

The results of this study showed that reprocessed catheters have cracks and roughness, which foster the retention of organic material with potential risk to the patient. Considering these results, the reuse of catheters should be discouraged by health institutions, avoiding any threat to the patient's health. In addition, this study contributes to promoting awareness in hospitals about the concerns with reuse of catheters in clinical procedures, about establishing new protocols for reprocessing catheters and about systematic surveillance of single-use utensils. The present study allows the reader to reflect on the questionable efficiency of reprocessing and number of points that should prompt institutes to police the reuse of all single-use devices.

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AdmiltonGonçalves de Oliveira: Designed the research plan, organized.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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