Sterilization of Paper During Crisis

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Abstract

Paper sheets represent one of the infection risk sources inside many institutions. The present study aimed to validate the sterilization efficiency of contaminated paper sheets with different indicator pathogens by gamma radiation or dry heat technique with retaining their structure. The results show that gamma irradiation at 6 kGy, 12 kGy, and 24 kGy can successfully kill Gram-positive bacteria like *Bacillus cereus* and *Staphylococcus aureus*, Gram-negative bacteria like *Escherichia Coli* and *Salmonella typhi,* and fungi such as *Candida albicans*. Moreover, dry heat at 100°C for 1 hour, 150 for 30 min, or 200 for 15 min can be successful in paper decontamination completely all tested species. Surprise, micrographs of scanning electron microscope (SEM) proved that a radiation dose of 6 kGy, dry heat at 100°C for 1 hour, 150 for 30 min or 200 for 15 min is suitable for paper sheets sterilization with maintaining their structure. Ultimately, dry heat or γ-radiation as simple, useful, effective, fast, safe, and inexpensive techniques. It may be used as a precautionary step inside educational institutions especially, during written examinations period to ensure safe life for academic members during biological pandemics like covid-19.

Key Points

The SEM technique was used to investigate the structure of the sterilized paper.

Using dry heating is easy and safer than gamma radiation in paper sterilization.

Ovens can be available in all institutions.

Introduction

The spread of the Covid-19 virus has an impact on the educational process around the world. Many schools and universities became increasingly closed because the Corona pandemic caused severe risks that reach death in some cases. Coronaviruses can remain infectious on surfaces for up to nine days at room temperature (Henwood 2020). When schools and universities reopen, paper sheets represent one of the infection risk sources in the academic community. It is necessary to find a proper technique for paper sterilization to protect academic colleagues from probable infections.

Sterilization is a process that effectively eliminates all pathogens such as viruses, bacteria, fungi, spore forms. Microorganisms vary widely in their resistance to disinfection. Bacterial spores have innate immunity. According to the relative scale of resistance (Fig. 1), the coronaviruses are the most sensitive to disinfection (William and Weber 2008). Enveloped viruses, under the influence of dry heating or gamma radiation, are more susceptible to inactivation than viruses without envelopes. The lipids building the envelope undergo peroxidation (Blázquez et al. 2019). Therefore, the dose of radiation eliminating bacterial spores will also be destructive to enveloped viruses.

The utilize of biological indicators is represented the most reliable, easy, and fast technique of sterilization control. These indicators are mentioned in the EN ISO 11138-1: 2017 standard concerning the sterilization of products (BSI 2017). The bioindicator systems contain spores of a nonpathogenic microorganism, suitable for a tested type of sterilization, with the highest resistance to the action of a distinct sterilizing agent (Kierat et al. 2020). On the other hand, different sterilization techniques depend on the material type (Singh et al. 2016). Coronaviruses can remain infectious on surfaces for up to nine days at room temperature (Henwood 2020). When schools and universities reopen, answer paper sheets represent one of the infection sources for the academic community. Antiseptic solutions, Autoclaves, or microwaves sterilization is not suitable for paper disinfection (Li et al. 2020). The Ultraviolet sterilization of paper must be achieved by one at a time because of UV cannot penetrate more than one. This technique should not be used to sterilize a large number of paper sheets.

Gamma sterilization is a cold sterilization technique for microbial inactivation for different materials. International standards for radiation sterilization ask for evidence of a minimum dose of 25 kGy induces irreversible structural changes in many materials (Karina et al. 2018). Radiation sources like Co-60 and Cs-137 can release high-energy electromagnetic gamma rays and effectively eliminates contaminating microorganisms (Silindir and Özer 2009). There are different opinions about the radiation dose to achieve the sterilization of paper sheets without damage. Gonzales *et al.* (2002) used 14.4 kGy to commercial papers and the resistance of the paper remains with no change (Gonzalez et al. 2002). Gamma radiation from three up to fifteen kGy showed no significant effects in the properties of irradiated paper (D'Almeida et al. 2009). However, gamma rays are very effective in penetrating and sterilizing paper, and the lack of gamma cells in our educational institutes makes this method unsuitable for paper sterilization.

A safe and comfortable decontamination method to sterilize paper sheets that are generally facing many challenges, such as maximally retaining the physical properties of paper and effectively decontaminate various types of pathogens with a distinct resistance to the sterilization method. Wow, dry heat sterilization is a safe, comfortable and effective technique that can achieve printed paper sterilization in a high number reach 2000 ones at a time with maximally retaining their structural properties. Additionally, an oven is an available device in all laboratories of educational institutes that makes this method the best one for paper sterilization.

Dry heat sterilization represents the oldest techniques used in sterilization. The heat at 70□C could kill a wide range of pathogens (Vieira and Pecchia 2018; Xiang et al. 2020). The actual sterilization time reaches an hour at a temperature of 160 to 170 0°C (Rashed et al. 2020). Moreover, the sterilization methods are used in the decontamination of viruses. Incubation at 50°C or 60°C for half-hour could inactivate viruses (Bertrand et al. 2012). A higher temperature is a safer option for the inactivation of SARS-CoV-2 (Xiang et al. 2020). Many coronaviruses are inactive after the following exposure times to temperatures: 90 minutes at 56°C, 60 minutes at 67°C, and 30 minutes at 75°C (Duan et al. 2003). Concerning the physical properties of heat sterilized paper, it was proved that using a 175 degrees Celsius improves the mechanical properties of the printing paper (Koubaa and Koran 2018).

The current study aims to find the minimum exposure time, heat, or radiation dose to sterilize paper sheets with maintaining their structural properties and the maximum exposure time, heat, or radiation dose that may be cause paper damage.

Materials And Methods

Contamination of paper

Gram-positive bacteria like *Bacillus cereus* ATCC- 12228 and *Staphylococcus aureus* ATCC-47077, Gram-negative bacteria like *Escherichia Coli* ATCC- 25922, and *Salmonella typhi* ATCC-15566 and *Candida albicans* ATCC- 10231 were used as references strains. The cultures of tested pathogens were prepared into nutrient broth medium overnight, the solutions of 104 CFU/ml of the tested strains were done in sterilized tap water using a hemocytometer slide.

After preparation of the microbial cultures solution, printing paper (3*3 cm) samples were treated by the prepared solutions using sterilized swaps. The treated paper sections were exposed to radiation or temperature treatments.

Sterilization methods

Paper sheets were sterilized by two different methods; gamma irradiation and dry heat. Further, the change in the structural properties was evaluated. In the first method, paper sheets (3 × 3 mm) were packed in Petri dishes and exposed to a gamma dose of 6 kGy,12 kGy, or 24 kGy. The radiation was applied at a dose rate, 125 Gy/min, using a Canadian Gamma Cell 40- Cesium 137 biological sources used, belonging to the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. The dose rate level for the paper (6, 12, and 24 kGy) at a dose of 0.6 Gy/sec.

In the second method, at a time, 500 answer paper sheets (printed paper) were exposed to high temperatures ranging from 100, 150, or 200 ℃ for different periods (15, 30, or 60 mints) at the Al-Nairiya University College laboratories, Hafr Al Batin University, Saudi El Arabia.

Microbial counts determination

The treated paper samples were divided to two groups, the first one was put into 10 ml sterilized water and vortexed. Paper samples were homogenized in water. From serial dilutions, 1 ml was inoculated on the surface of nutrient agar plates. After incubation, the number of grown colonies was counted and recorded. The other group was putted onto the nutrient agar plate surfaces and incubated for 48 h at 37 ℃, and the growth of microbes was noted.

Scanning Electron Microscope (SEM)

The morphology of the sterilized paper was detected through scanning electron microscopy (SEM) using a field-emission scanning electron microscope (Model, Quanta 250 FEG; field-emission Gun, JEM2100,

Jeol, Japan).

Results

Gamma radiation sterilization

Data in **Table** (1) and **Fig**. (2) showed that γ-radiation at all tested doses (6 kGy, 12 kGy, or 24 kGy) is an effective technique in the sterilization of paper sheets contaminated with Gram-positive bacteria like *Bacillus cereus* and *Staphylococcus aureus*, Gram-negative bacteria like *Escherichia Coli* and *Salmonella typhi,* and fungi like *Candida albicans* .

Dry heating sterilization

The effectiveness of dry heating in the sterilization of paper samples depending on the type of tested pathogenic species, temperature degree, exposure period. The dry heating sterilization using temperature at 100, 150, and 200 ℃ for 15 mint markedly reduced the number of *Bacillus cereus* by 99.0%, 99.9%, and 100%, *Staphylococcus aureus* by 99.0%, 99.6% and 100%, *E. Coli* by 97.2%,97.7%, 100%*, Salmonella typhi* 98.3%,98.6 and 100%and *Candida albicans* by 97.1%, 97.6%, and 100%, respectively compared to the corresponding control (**Table** 2 and **Fig**. 3).

Data represented in **Table** (2) and **Fig**. (4) showed that temperature at 100 ℃ for 30 mint inhibitedthe growth of *Bacillus cereus* by 100% and the number of *Staphylococcus aureus, E. Coli, Salmonella typhi,* and *Candida albicans* by99.9% for each mentioned pathogen compared to the corresponding controls. Moreover, 150 ℃ or 200 ℃ for 30 mint can destroy all tested pathogenic microorganisms contaminated paper. Besides, dry sterilization using 100, 150, or 200 ℃ for one hour is an effective method for killing all tested pathogens.

Effect of gamma radiation on paper structure

The scanning electron microscope **(**SEM) technique was used to study the structure and morphology of the sterilized paper sheets. Each SEM micrograph in **Fig. 5** showed the changes in the paper structure after gamma sterilization. Control samples of paper sheets have a high-density of intertwined cellulose fibers, different shapes and sizes, and calcium carbonate agglomerates **(Fig. 5a)**. On the other hand, gamma radiation at 6 kGy caused flatness of the cellulose micro-fibrils, providing a larger surface area **(Fig. 5b)**. In addition, a high-density structure of intertwined cellulose fibers and calcium carbonate agglomerates was observed. However, the irradiated paper sheets with 12 kGY showed a decline in binding joints resulted in the reduction of inter-fiber forces **(Fig. 5c)**. Furthermore, the high dose of γ-radiation (24 kGy) resulted in a severe degree of hornification **(Fig. 5d)**.

Effect of dry heat sterilization on paper structure

In **Fig. 6**, SEM images of the dry heating paper sheets for one hour were examined and compared to unheated ones (**Fig. 6a**). The results showed that the treated sheet with 100°C attained a slight reduction

in bonding between cellulose microfibers (**Fig. 6b**). However, the deformation of cellulose microfiber was detected in the microstructure images of the dry heating paper sheets at 150 °C or 200 °C (**Figs. 6c and 6d**). Only the 200 °C dry heated sheets gained a severe heterogeneous microstructure as well as a yellow color.

Discussion

The sterilization of paper samples depending on the type of tested pathogenic species, type of treatment (radiation or heating), and exposure period. In this study, γ-radiation represents an effective technique to sterilize the pathogenic contaminated paper sheets. These results may be due to gamma radiation generates free radicals that react with biological molecules. DNA is highly susceptible to the effects of radiation (Kuefner et al. 2015). Damage in DNA molecules eventually leads to cell death (Sage and Shikazono 2017; Harrell et al. 2018).

According to the current work, the results indicate that dry heat can kill a wide range of pathogens. Dry heating sterilization is the best method for paper sterilization. The main reason is due to its low penetration which retained physical properties of paper. Moreover, the use of an oven is considered convenient and economical (Xiang et al. 2020). Wet sterilization is not suitable for paper disinfection because of its high penetration (Li et al. 2020), the higher temperature and steam may affect the paper structure than dry heat.

The scanning electron microscope (SEM) technique to investigate the structure and morphology of the dry heat or gamma-radiation sterilized paper sheets. Gamma radiation caused flatness of the cellulose microfibrils, a high-density structure of intertwined cellulose fibers and calcium carbonate agglomerates, a decline in binding joints resulted in the reduction of inter-fiber forces or a severe degree of hornification. These results may be due to a decline of the water-holding potential of cellulose microfibrils or dry conditions leading to lower swelling of the microfibres, density, and strength properties of paper sheets (Salmén and Stevanic 2018). Furthermore, the strength properties of cellulose fibers were due to the increased density of molecular cross-linking, depended on the gamma-rays dose. After 10 kGy, the strength properties of cellulose fibers decreased gradually with the γ-ray dosage (Hoque et al. 2017).

The treated paper sheet with 100°C for one hour attained a slight reduction in bonding between cellulose micro-fibrils. However, the deformation of cellulose microfiber was detected in the microstructure images of the dry heating paper sheets at high temperature especially the 200°C dry heated sheets which gained a severe heterogeneous microstructure as well as a yellow color. These changes may be due to the paper component were susceptible to dryness condition and losses of the swelling ability of micro-fibrils. In the same concern, the loss of swelling capability of cellulose micro-fibrils upon drying is due to the closure of the pores in the cell wall. When cellulose microfibers were dried in contact, they essentially could be healed together and become one (Hubbe 2014).

Ultimately, our study proved the possibility of paper sheets sterilization without structural damage. Sterilization of paper sheets with gamma radiation at 6 kGy or temperature at 100 ℃ for 1 hour can be

used as the effective treatments to kill or destroy pathogens probably attached paper sheets. Dry heat or gamma radiation is used as simple, effective, fast, safe, and inexpensive techniques to ensure safe life of a for different disease infections. During biological crisis like Covid-19, educational institutions must be considering the risk of probably contaminated paper especially, with the reopening of schools and universities.

Declarations

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Author contributions

F.H.A., conceptualization, methodology, writing review and editing, and H. A. H., material preparation, investigation, wring the first draft.

Availability of data and materials data are available upon request.

Code availability Not applicable.

Ethics approval Not applicable.

Conflict of interest The authors declare no conflict of interests.

Consent to participate Not applicable.

Consent for publication Authors gave their consent for publication of this study.

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Tables

Table 1 The efficiency of γ-radiation at different doses in the sterilization of pathogenic contaminated paper.

Treatments		Average no. of organisms				Inactivation of test organism %
Tested organism	γ-radiation	Control	6 kGy	12 kGy	24 kGy	
Gram-positive bacteria	*Bacillus cereus*	$10x10^6$	0.0	0.0	0.0	100
	Staphylococcus aureus	$8x10^7$	0.0	0.0	0.0	100
Gram-negative bacteria	*Escherichia Coli*	$9x10^6$	0.0	0.0	0.0	100
	Salmonella typhi	$12x10^6$	0.0	0.0	0.0	100
Fungi	*Candida albicans*	$8x10^6$	0.0	0.0	0.0	100

Table 2 The efficiency of dry heat at different periods in the sterilization pathogenic contaminated paper.

Treatments			Average no. of organisms				Inactivation of tested organism %		
Time (mint)	Temperature (°C) / Tested organism		Control	100 °C	150 °C	200 °C	100° C	150° C	200° C
15 mint.	Gram-positive bacteria	*Bacillus cereus*	$10x10^6$	$6×10^3$	$5×10^3$	0.0	99.0	99.9	100
		Staphylococcus aureus	$8x10^7$	$30×10^4$	$27×10^4$	0.0	99.0	99.6	100
	Gram-negative bacteria	*Escherichia Coli*	$9x10^6$	$25×10^4$	$21×10^4$	0.0	97.2	97.7	100
		Salmonella typhi	$12x10^6$	$20×10^4$	$17×10^4$	0.0	98.3	98.6	100
	Fungi	*Candida albicans*	$8x10^6$	$23×10^4$	$19×10^4$	0.0	97.1	97.6	100
30 mint.	Gram-positive bacteria	*Bacillus cereus*	$10x10^6$	0.0	0.0	0.0	100	100	100
		Staphylococcus aureus	$8x10^7$	$7×10^2$	0.0	0.0	99.9	100	100
	Gram-negative bacteria	*Escherichia Coli*	$9x10^6$	$5×10^2$	0.0	0.0	99.9	100	100
		Salmonella typhi	$12x10^6$	$3.5×10^2$	0.0	0.0	99.9	100	100
	Fungi	*Candida albicans*	$8x10^6$	$4×10^2$	0.0	0.0	99.9	100	100
60 mint.	Gram-positive bacteria	*Bacillus cereus*	$10x10^6$	0.0	0.0	0.0	100	100	100
		Staphylococcus aureus	$8x10^7$	0.0	0.0	0.0	100	100	100
	Gram-negative bacteria	*Escherichia Coli*	$9x10^6$	0.0	0.0	0.0	100	100	100
		Salmonella typhi	$12x10^6$	0.0	0.0	0.0	100	100	100
	Fungi	*Candida albicans*	$8x10^6$	0.0	0.0	0.0	100	100	100

Figures

Figure 1

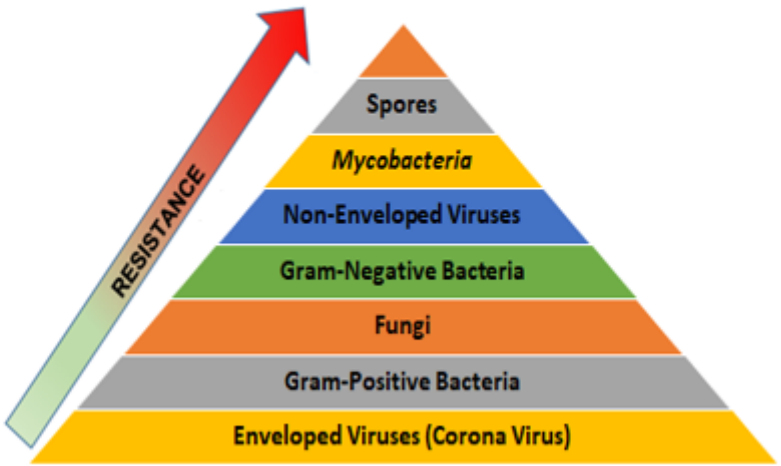

Figure 1

Resistance of microorganisms to the sterilization (William and Weber 2008).

Figure 2

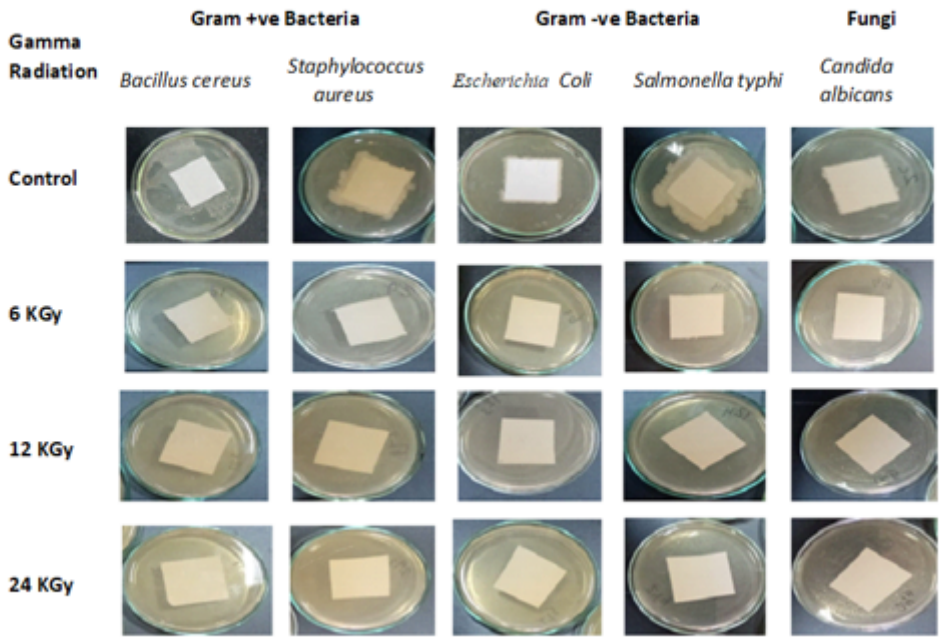

Figure 2

The efficiency of γ- radiation in the sterilization of pathogenic contaminated paper.

Figure 3

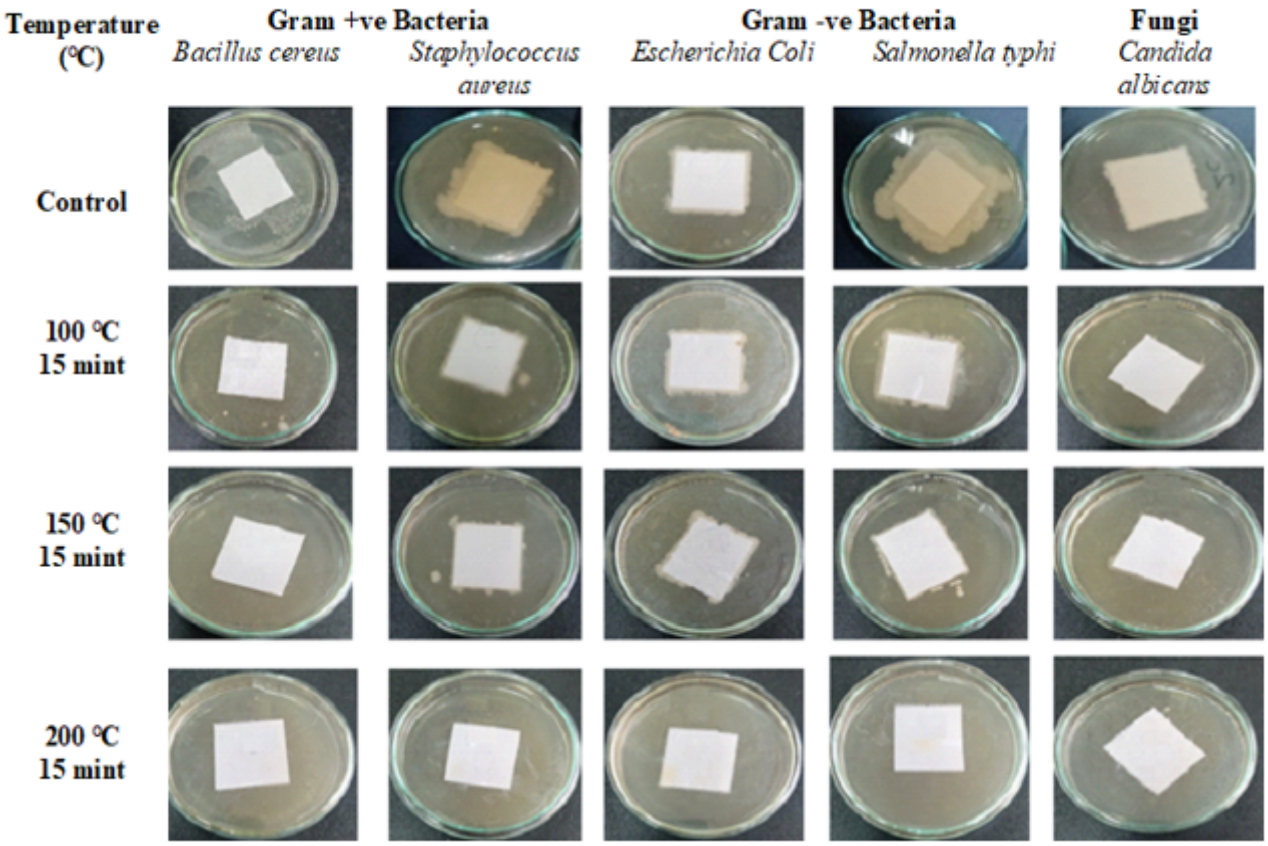

Figure 3

The efficiency of different temperatures for 15 mint in the sterilization of pathogenic contaminated paper.

Figure 4

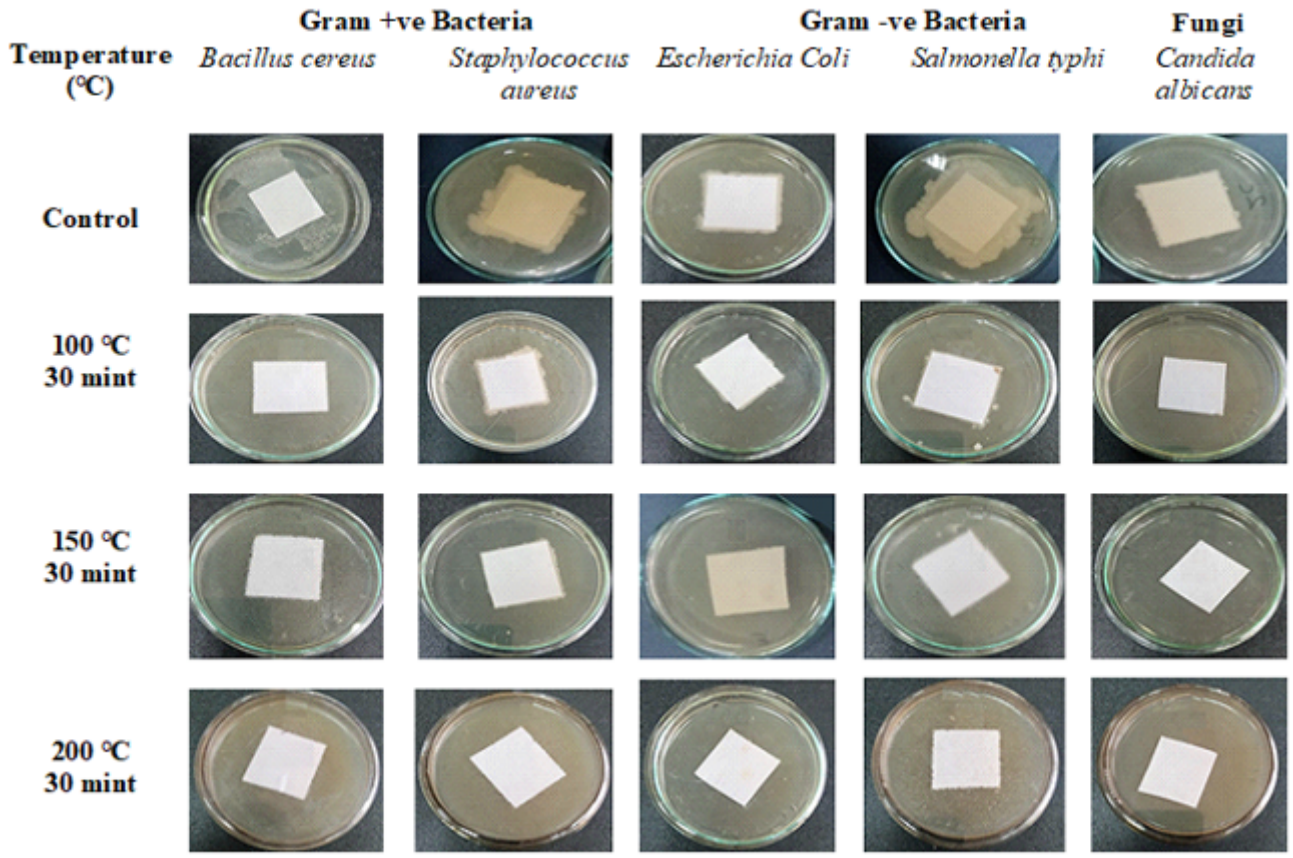

Figure 4

The efficiency of different temperatures for 30 mint in the sterilization of pathogenic contaminated paper.

Figure 5

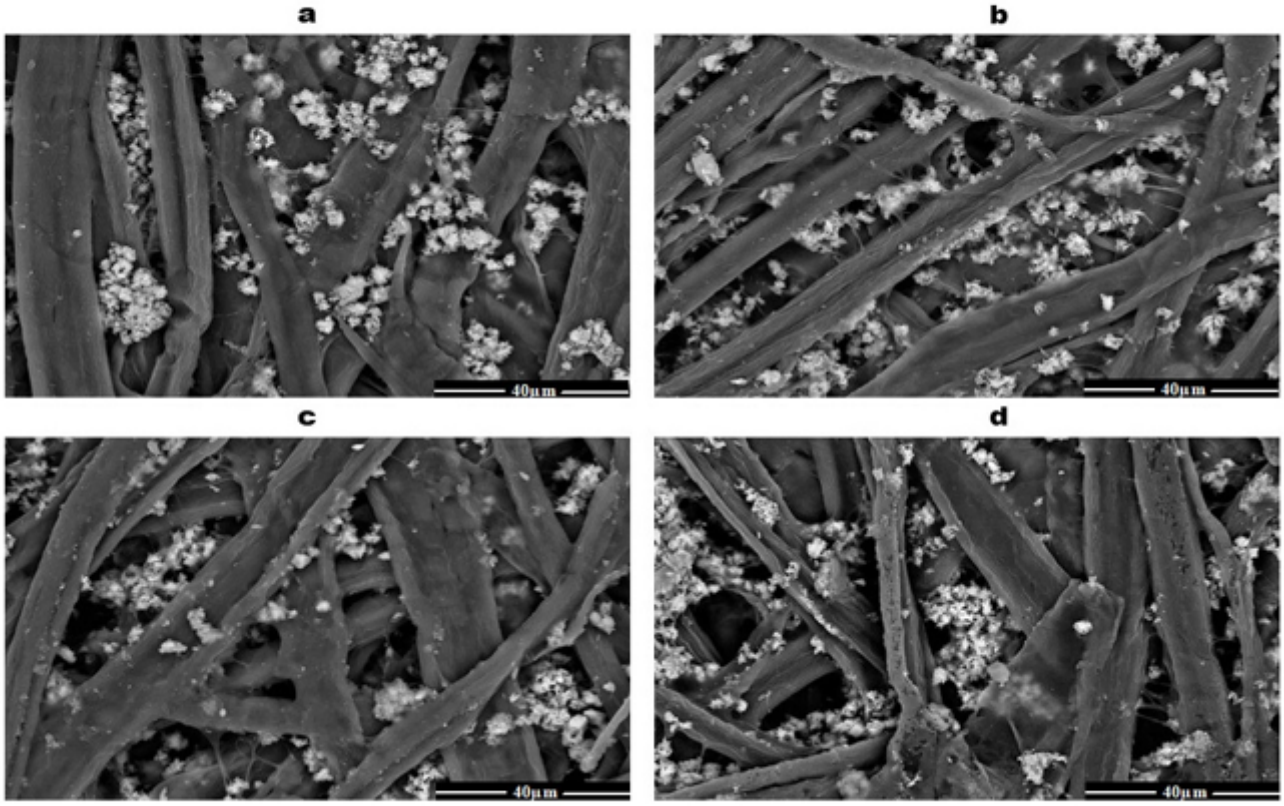

Figure 5

SEM micrograph of γ-radiation effect on paper structure. Control (a), 6 kGy (b), 12 kGy (c), and 24 kGy (d)

Figure 6

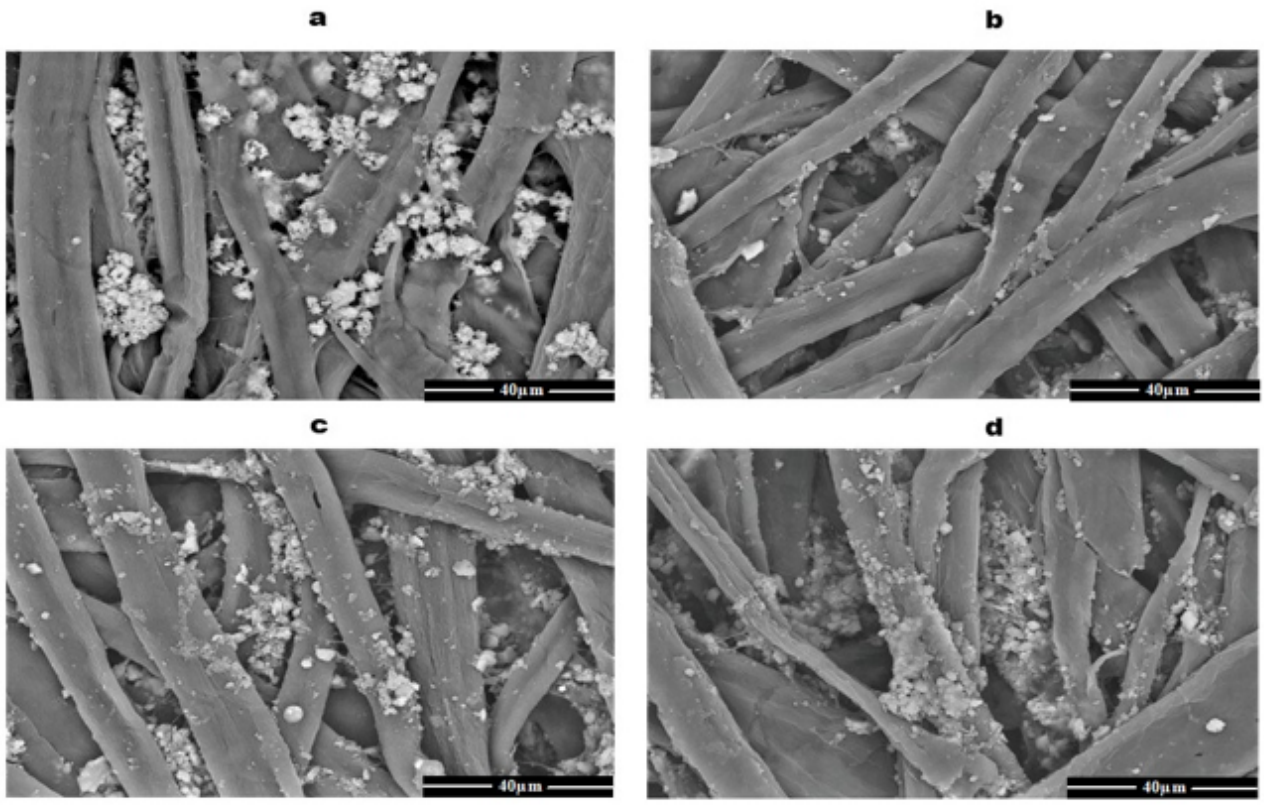

Figure 6

SEM micrograph of dry heat effect on paper structure. Control (a), 100 °C (b), 150 °C (c), and 200 °C (d)