COMMUNICATIONS IN
DEVELOPMENT AND ASSEMBLING OF TEXTILE PRODUCTS

Influence of polyester fabric with infrared emissive additives on cell metabolism

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INFO

CDAPT, ISSN 2701-939X
Peer reviewed article
2022, Vol. 3, No. 2, pp. 104-114
DOI 10.25367/cdatp.2022.3.p104-114
Received: 14 May 2022
Accepted: 26 May 2022
Available online: 3 September 2022

ABSTRACT

Functional fabrics with effects of influencing the metabolism and energy supplying are of great interest and may extensively be applied in wellness and sport apparel. In this work, three different combinations of additives such as silicon oxide doped with 10% of iron oxide III, 10% of graphene and 10% calcium hydroxyapatite were prepared, each combination was compounded separately with polyester, and pellets were extruded with additive weight around 4%. Melt spun multifilament yarns (130D/48f) were produced from the prepared pellets. Further, three knitted fabrics from a yarn with additives and one control fabric from the yarn without additives were developed for comparison and analysis. Infrared emissivity test result showed that fabrics with functional additives have significantly higher effective emissivity (0.997 to 1.006) than the reference fabric (0.909) for wavelength 5-14 μm. Moreover, spectral emissivity of fabrics with additives are relatively high at peak human IR emission wavelength (8-14 μm). As a key signal molecule that is involved in certain physiological pathways, nitric oxide (NO) generation was assessed by co-culturing with human skeletal muscle cells (HMSCs). It is observed that selected additives in the fabric lead to 15%-40% increase of nitrite levels in muscle cells after 24 and 72 hours of exposure and the best among them were graphene containing additives. Besides, it is also discovered that additives in the fabric increase mitochondrial biogenesis, which is proved by the increase of mitochondrial copy number by a factor of 1.25. The mitochondrial biogenesis may be a possible pathway activated by nitric oxide and potentially accelerate the energy expenditure. The observations in the cell study indicated the potential biological effects of the fabric with selected functional additives.

Keywords
Functional apparel
Infrared ray
Nitric oxide
Mitochondrial biogenesis

1 Introduction

In recent years, functional textile materials have attracted more attention among manufacturers and researchers when compared to the ordinary textile materials and apparels because of their advanced characteristics and more wellbeing benefits [1,2]. Among various functional materials, apparels or fabrics with bioceramic that could emit far-infrared rays (FIR) have attracted more and more interest [3,4]. Many products using fabric with far-infrared (FIR) emitting bioceramic have been developed and are available in the commercial market including apparels, underwear, footwear, bedding, mattresses, and pet products [5,6]. Apparels or clothing materials next to the human skin with FIR-emitting ceramic could absorb the infrared radiation released by the body in the wavelength range of 6-14 μm and reflect it back to the body [7]. This reflection can bring various positive effects to the human body such as an increase of blood flow, promote tissue oxygenation, enhance sport performance and improve recovery etc. [8,9]. Hence, these kinds of materials with the above mentioned wellbeing benefits make more promising products, especially in the human wellness and sports related developments [10]. Physical exercise and sport activities cause a lot of changes and adaptations to the human body and are influenced by application of special garments.

There are some explorations to use far infrared rays in human wellness and the area of exercise and sport. The benefits include thermo effects, increasing blood flow, body weight management, pain reduction and improved sleep quality [11-13]. The application in the sport area showed an improved performance, shorter recovery time after an exercise, reduced muscle soreness after an exercise and reduced oxygen consumption during the low intensity exercise [8].

Inorganic oxides such as Al_2O_3, SiO_2, MgO, TiO_2 and ZnO and their mixtures have been applied as infrared emitting bioceramic. Graphene has superior thermal conductivity and excellent far-infrared emission [14]. Previous studies showed that the far-infrared energy emitted by a graphene containing device could be used as a novel non-invasive therapeutic tool for tumor treatment. In a breast cancer cell xenograft model, graphene far-infrared emitter treatment reduced the rate of tumor growth by 42% [15]. Their study demonstrated that the stronger penetrating ability of the far-infrared radiation generated by the graphene leads to superior therapeutic effects.

Currently, the mechanism of far-infrared ray on the human body is still under investigation. Previous studies indicate that infrared rays may possibly influence nitric oxide production [16,17]. Nitric oxide is an important signal molecule, lead to downstream physiological effects, including vasodilation, modulation of mitochondrial function, modulation of glucose metabolism and decreasing inflammation. These pathways are closely related to the human metabolism and health, as well as sport and exercise performance. In our study, three types of inorganic additives, containing iron oxide III, calcium hydroxyapatite and graphene, were incorporated into fabrics. Graphene is a well-known material which absorbs energy in a large wavelength range and emits IR radiation [14]. Apatites are also popular basements in composite materials, which can reflect and emit waves in the infrared range [18,19]. Iron oxide was included to the study as an antagonist to additives, being known as material with a high ability to absorb energy in the large wavelength range [20]. We investigated their biological effects in human skeletal muscle cells, especially on nitric oxide production and their influence on mitochondria.

2 Materials and methods

2.1 FIR emissive additives

Quartz powder was purchased from Merck, particle size 45 μm, graphene was provided by the company 2DM Solutions, general purpose grade GP002-20, average specific area: 90 m^2/g, average lateral size: 1-3 μm; red iron oxide, a pigment with particle size 50 μm was obtained from Earth Pigments; calcium hydroxyapatite mineral in a raw form was purchased from Mikon Co. All materials obtained from corresponding suppliers were used as received.

Three additives were fabricated and tested in the study. Additive 1 contains the mixture of silicon oxide doped with 10% of iron oxide III, additive 2 contains mixture of silicon oxide and 10% of calcium

hydroxyapatite, while additive 3 contains mixture of silicon oxide and 10% of graphene. Each additive was prepared by mixing of 90% of silicon oxide (quartz powder) and 10% of iron oxide/hydroxyapatite/graphene accordingly in the horizontal planetary ball mill (Biobased). Stainless steel pots (500 ml) with alumina balls (3 mm) were used. Ratio of material to balls was 3:1 by weight (100 g of material, 300 g of balls). Material was milled for 75 min with changes of milling direction every 5 minutes at 60% of maximum speed.

Field emission scanning electron microscope (FESEM, JSM-7600F, JEOL) operated at the accelerating voltage of 5 kV with EDX function was used for the evaluation of shape, size and semi-quantitative analysis of the composite powders obtained after milling.

2.2 Development of yarns and fabrics

Powder after ball mill was used for the preparation of polyester composite pellets in twin screw extruder compounder (PSHJ-20, Jiangsu Xinda Tech Limited, China) at the speed of 6.5 kg/h with the temperature profile of five heating zone as 245, 248, 250, 250, 260 °C. The extruder compounder has two feeders: the main feeder for polymer pellets and the second feeder for powder. Targeted amount of inorganic additive in pellets were 5 wt%. Pellets with additives were dried at the oven at 110 °C with dehumidifier overnight before the yarn fabrication.

Multifilament yarn was produced using a melt spinning machine (FET 100). The multifilament yarns were spun using spinneret with 48 holes (0.6 mm hole size) at the speed of the melt pump of 17 rpm, godets speeds of 650, 1200, 1500 m/min and a winder speed of 1500 m/min. The temperature profile was set to 245, 275, 280, 283, 285 °C for a screw barrel and 285 °C for a melt pump. The obtained yarn has a linear density of 130 denier.

Single jersey knitted fabric was developed using customized 24 gauge, 6-feeder, 16-inch (Cylinder Dia) circular knitting machine supplied from Anytester Co. Ltd.

2.3 Infrared emissivity, spectral transmittance, and diffuse reflectance measurement

The infrared emissivity spectrum was measured at 21.3 °C using an infrared spectrometer and black body at a wavelength of 4-16 μm by an external standard institute (National Institute of Measurement and Testing Technology, NIMTT, China). Additionally, the integral far infrared emissivity was measured using a benchtop HOTECH far infrared emissivity analysis system EMS302M at a wavelength of 5-14 μm.

The spectral transmittance (%T) and diffuse reflectance (%R) were measured by a UV-Vis-NIR spectrophotometer (Lambda 950, Perkin Elmer) with a 150 mm integrating sphere over the range of 0.2-2.5 μm, and a Fourier transform infrared (FTIR) spectrometer (Nicolet iS-50, Thermo Scientific) with a gold integrating sphere (PIKE Technologies) over the range of 2.5-16 μm. The spectral absorptance/emissivity (%A) was calculated by %A = 100% - %T - %R.

2.4 Cell culture

Human skeletal muscle cells (HSMCs) were purchased from Lonza Bioscience and cultured in skeletal muscle cell growth medium (SKGM) (Lonza, US). HSMCs were seeded on 12-well culture plates at a density of 1×10^5 for 24 hours before subjecting to the treatment. The fabrics without and with additives were pre-sterilized with isopropanol and 70% ethanol, and then placed in the insert and co-cultured with HSMCs in a 37 °C, 5% CO_2 humidified incubator.

2.5 Measurement of nitrite/nitrate levels

After co-cultured with cells for 24 hours and 72 hours, cells were collected and nitrite/nitrate levels was measured using Parameter™ Total Nitric Oxide and Nitrate/Nitrite Assay (RnD systems, Cat #KGE001). This assay determines nitric oxide concentrations based on the enzymatic conversion of nitrate to nitrite

by nitrate reductase. The nitrite level was first determined, followed by an enzymatic conversion of nitrate in the cells to nitrite, and then nitrate level was determined by subtracting pre-determined nitrite level from the total amount of nitrite level after the enzymatic conversion.

2.6 Nitric oxide synthase assessments

The concentration of inducible nitric oxide synthase (iNOS) was determined using human iNOS ELISA kit (Abcam, Cat# ab253217) according to a manufacturer's protocol. Sample values were then read off the standard curve. The amount of iNOS was normalized to total protein level and results were expressed in the relative level.

2.7 Mitochondrial DNA copy number measurement

Mitochondrial DNA copy number was assessed by real time PCR. Briefly, total RNA extraction was performed using E.Z.N.A total RNA kit (Omega Bio-Tek) and used as a template for complementary (c)DNA synthesis using iScript reverse transcription kit (Bio Rad) according to the manufacturer's protocol. Real time PCR was performed using cDNA as templates on Bio-rad CFX Connect Realtime PCR system using default setting for SYBR green reaction. Primers for POLG2 (DNA Polymerase Gamma 2) encoding the subunit of mitochondrial polymerase gamma was used. Gene expression levels were calculated by ΔΔCT and normalized using GADPH as endogenous control. The primer sequences of POLG2 were as follows: POLG2-F: 5'-CCGGAGCTGTTGACGGAAA-3' and POLG2-R: 5'-TTCCACTTAGGAAATGCCTTCTC-3'. GADPH-F: 5'-GTCTCCTCTGACTTCAACAGC-3' and GADPH-R: 5'-ACCACCCTGTTGCTGTAGCC-3'.

3 Results

3.1 Particle size and composition of additives

Properties of obtained additives were studied. The median particle size after milling was in the range of 5-12 μm for all additives. Particles of additive 1 have a median size of 6.12 μm, while the ones of additives 2 and 3 have median sizes of 4.46 μm and 4.45 μm, respectively. The EDX function of the SEM has shown the following semi-quantitative compositions of composite-additives (Table 1).

Table 1. Compositions of the three additives.

	Additive 1	**Additive 2**	**Additive 3**
Composition (wt%)	Si: 36.7%	Si: 38.8%	Si: 35.1%
	Fe: 4.4%	O: 55.2%	O: 55.7%
	O: 56.1%	C: 0.8%	C: 9.2%
	C: 2.8%	P: 1.4%	
		Ca: 3.8%	

3.2 Spectroscopy measurements and emissivity

Spectroscopy has shown difference in transmission and absorption in different wavelengths among all three additives. It can be clearly seen from Figure 1 that all additives possess one main peak of absorption, which shifts slightly among all samples.

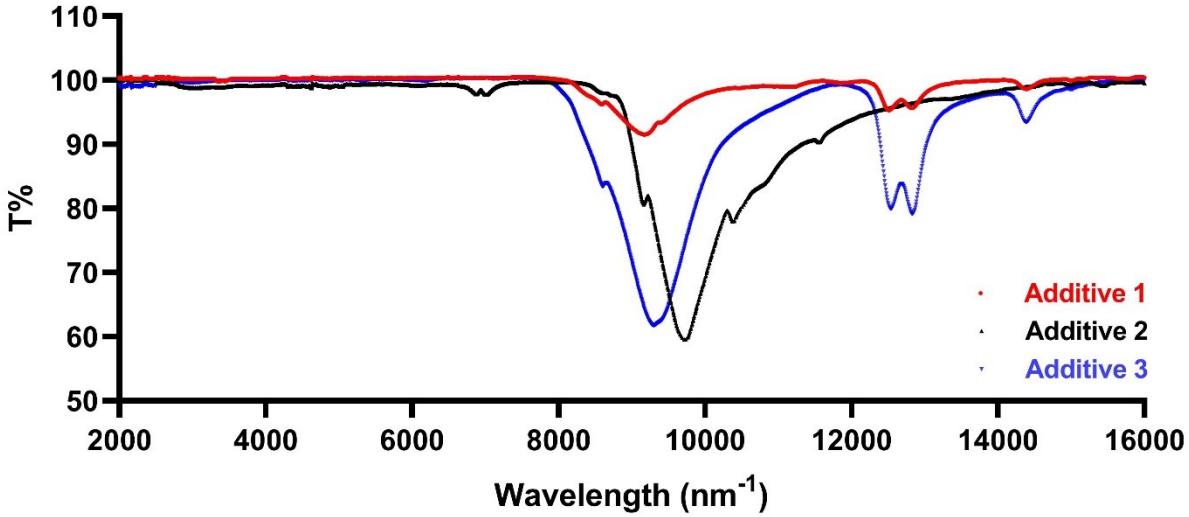

Fig. 1 Transmittance of three additives in the wavelength range from 2-16 μm.

Spectra of all additives contain a similar main peak at 9.272, 9.713 and 9.162 μm for additives 1, 2 and 3, respectively. Additive 1 gave small additional peaks at 12.516, 12.796 and 14.378 μm. Additive 3 also contains additional peaks at 12.531, 12.812 and 14.378 μm. Peaks mostly come from silicon oxide, but differences could be explained by the presence of doped elements.

Data related to emissivity of prepared additives and fabrics which contain about 4% of additives (measured by TGA in air) in the range of 5-14 μm is presented in Table 2.

Table 2. Emissivity of individual powders and polyester fabric with powder at 5-14 μm (relative to the black body).

Samples	Emissivity of powders	Emissivity of fabric
Additive 1	0.990	0.997
Additive 2	0.998	0.995
Additive 3	1.001	1.006

Additive 3 doping with a graphene has the highest increase in IR emissivity compared with the emissivity of black body. From the far IR emissivity spectrum (Figure 2), the emissivity in the range 4-16 μm was enhanced. The area under the curve (AUC) was increased by 2.56% for additive 3 compared with the blank sample without additives. The emissivity is especially enhanced in the range of 8-14 μm, which corresponds to the peak area of human infrared emission, increasing by 2.8% for additive 3 compared with the blank sample.

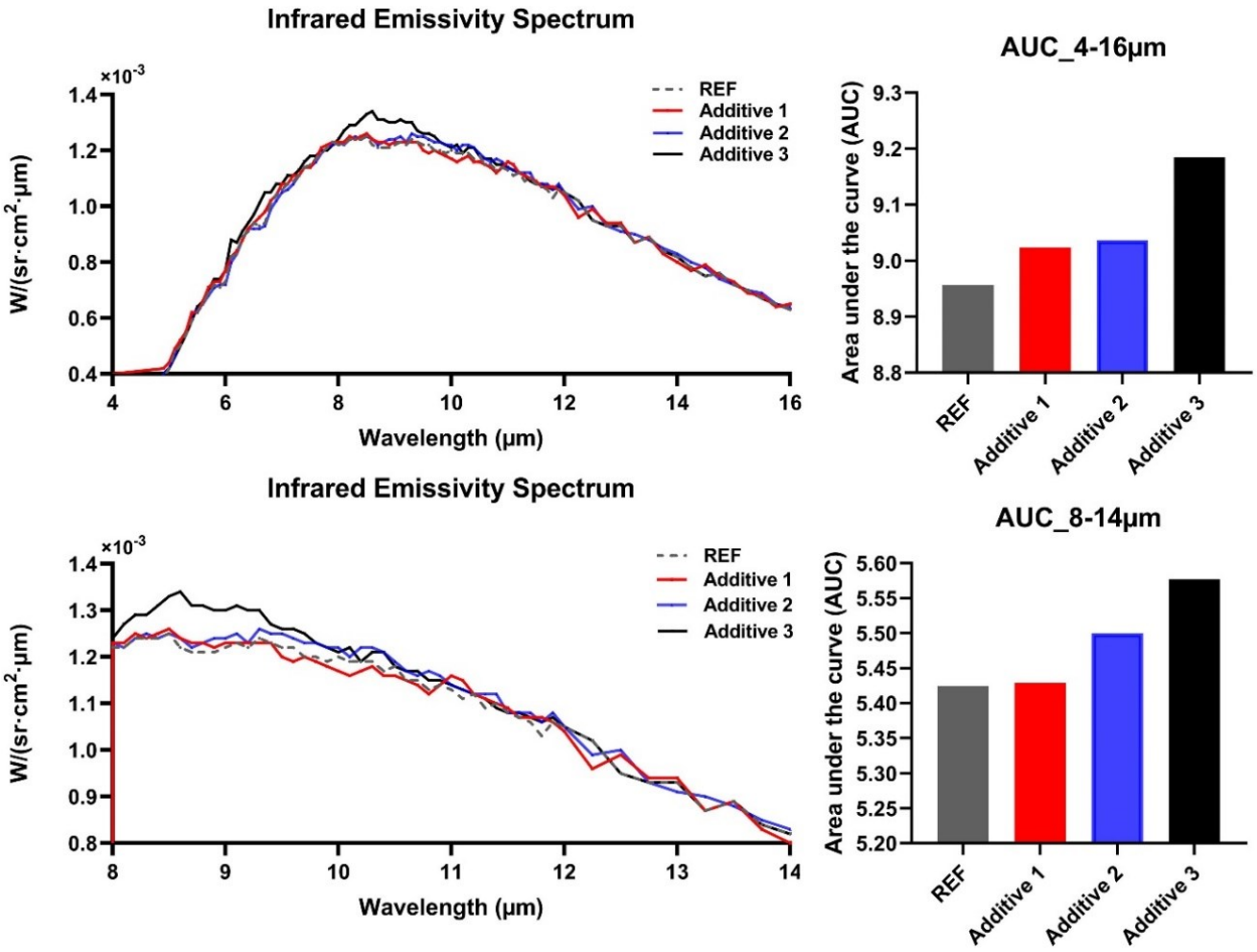

Fig. 2 Infrared emissivity spectrum of fabrics with inorganic additives in the ranges of 4-16 μm and 8-14 μm. The area under the curve (AUC) was calculated for each curve.

3.3 Effects on Nitric oxide production and mitochondrial copy number of HMSCs

The influence of additives on the nitric oxide production was evaluated in the HMSCs. Since NO is relatively unstable, we measured the metabolic products nitrite and nitrate in the study. After co-culturing for 24 hours, the nitrite levels of all 3 additive groups were higher than that of the control group. This was noticeable especially for additive 3 group, which showed significant higher amount of nitrite (43%) than the control group (Figure 3). Additive 3 group also showed consistent higher amount of nitrite at 72 hours, while additives 1 and 2 showed a slightly lower amount. Nitrite is converted into nitrate as another stable store of NO. To generate nitrite, nitrate needs to be converted into nitrite and then into NO. Nitrate level in all groups were similar in all three additives groups.

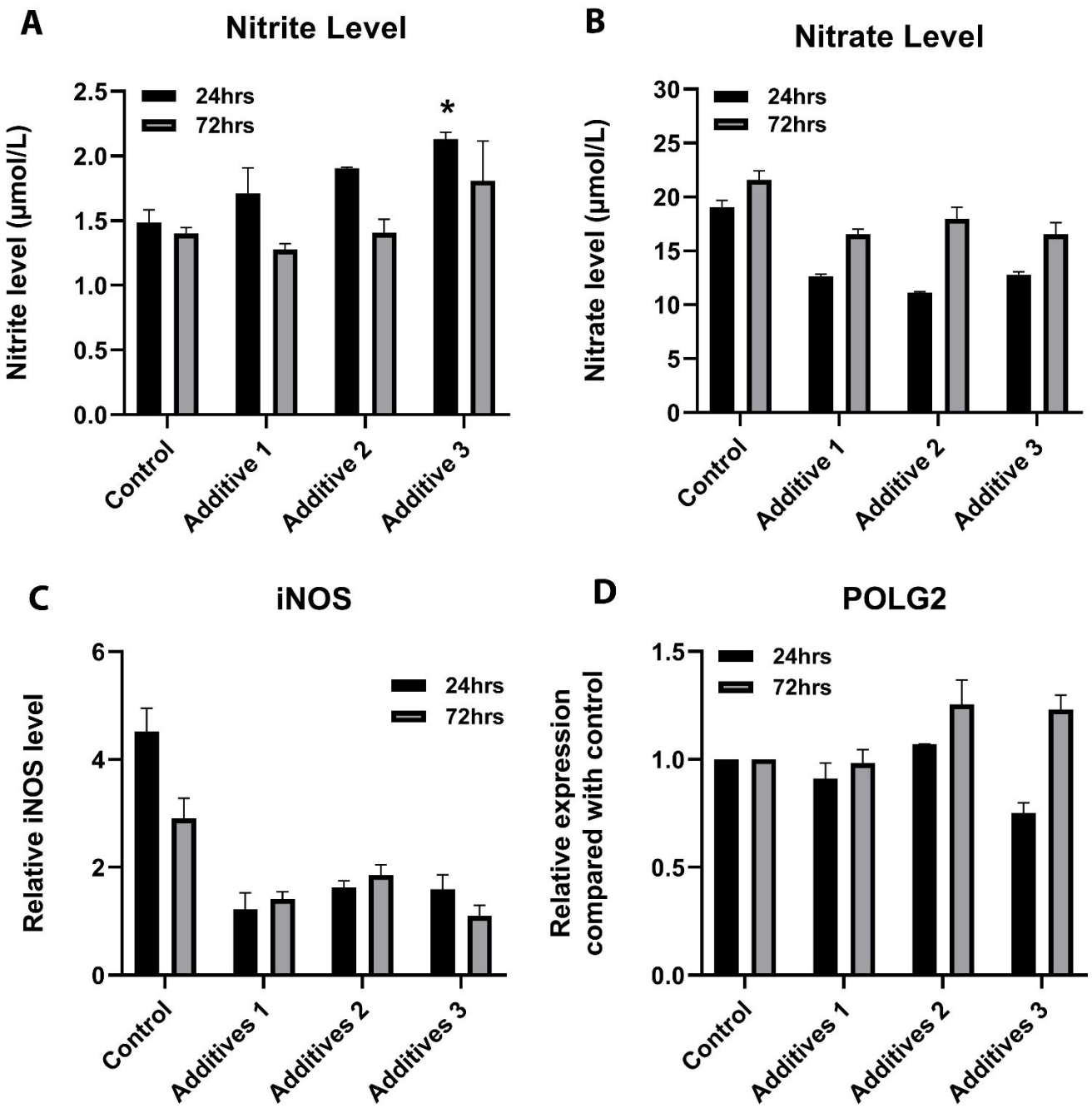

*Fig. 3 Nitrite (A) and nitrate (B) level of HMSCs cultured with the fabric without additive (control), fabrics with 4% of additive 1, 2 and 3; (C) relative amount of inducible nitric oxide synthase (iNOS) to the total protein level; (D) relative expression level of mitochondrial biomarker POLG2 (indicating the mitochondrial copy number), (*p < 0.05, t-test).*

We also measured the production of iNOS, which is closely related to the inflammation in the muscle cells. It could be seen that the iNOS level was even lower in additive groups. The result showed the increase in the nitric oxide production in the additive groups was not caused by inflammation.

Additive 1 showed similar level of expression as the control group. Compared with the control group, additive 2 showed an increase of mitochondrial copy number after 24 hours of culturing, while a similar higher level of expression was observed (1.25-fold change) after 72 hours of culturing. For additive 3 group, the mitochondrial copy number showed an increase from 24 hours to 72 hours, and the higher expression was observed after 72 hours of culturing (1.23-fold change) as compared to the control group.

4 Discussion

In general, bioceramic contains inorganic oxides emitting FIR radiation in the wavelength of 4-16 μm. In our study, incorporation of inorganic additives further increased the infrared emissivity in the above wavelength range, especially in the range of 8-14 μm corresponding to the peak area of human infrared emission. Yu et al. (2012) measured the infrared radiation spectrum of human body using a high precision spectrometer in the range of about 1-16 μm [21]. Over 50% of highest infrared emissivity were observed between 8 μm and 16 μm, while two peak areas of about 10-14 μm were observed. In another study using a human hand as an infrared light source [22], a peak area of emissivity in the range of 8-14 μm was detected. In our study, we found the major increase of additive group and control also fells on this range, overlapping with the peak region of the human body. The absorption efficiency of far-infrared rays depends on the degree of matching between the emission property of far-infrared ray and absorption property of the subjects that receive the irradiation. Good absorption efficiency could overcome the poor penetration problem faced by traditional far-infrared [15]. Therefore, the matching of FIR emission of our additives greatly increased the absorption rate, further influencing certain pathways in the human body.

Relevant studies to explore the health benefits in human trials have been performed. York et al. (2009) tested socks with FIR bioceramic on 55 patients with foot pain and observed pain reduction [23]. Silva et al. (2009) also observed pain relief and an improved sleeping quality in their study on patients with pain and poor sleeping quality [3]. Ko et al. (2002) showed FIR gloves could improve the blood circulation in Raynaud's patients [24]. In the application of sport wear, FIR apparels were found to have benefits including improved sport performance and reducing post-exercise muscle soreness in physically active population or athletes. Decreased energy expenditure and increased oxygen availability were also found in previous studies. Additional benefits for athletes include low bacterial growth and less foot sweating of FIR socks [6].

In vitro cell studies and *in vivo* animal studies further investigated the benefits and could provide some clues for possible mechanism of FIR emitting bioceramic. Leung et al. (2009) found FIR emitting bioceramic material increased nitric oxide production in different cell models [25,26]. Park (2013) showed far-infrared radiation increased Ca^{2+} mobilization and then activated endothelial nitric oxide synthase, finally increasing nitric oxide production [17]. Similar effects of FIR bioceramic on the nitric oxide production were also observed in both cell study and mice model [27]. As an important regulator of mitochondrial function, nitric oxide stimulates mitochondrial biogenesis through a pathway including activation of soluble guanylatecyclase (sGC), the production of cyclic guanosine monophosphate(cGMP) and peroxisome proliferator activated receptor c coactivator 1a (PGC-1α) [28]. Mitochondria play a central role in the energy metabolism. Increasing of mitochondrial biogenesis will increase the metabolic rate, energy expenditure, and fat utilization.

In our study, we analyzed the nitric oxide production and mitochondrial biogenesis. We found that the additive groups induced a higher nitrite production as soon as after 24 hours of treatment. The increase of nitric oxide was not related to the inflammation reaction, as demonstrated by the low amount of iNOS production observed in the additive groups. There is only a low amount of iNOS in normal human muscles cells in normal conditions, the increased expression of iNOS could be found during inflammation. Nitric oxide could be produced by nitric oxide synthase, or via a nitric oxide synthase-independent way activated by ion channels in the cells [29]. This process involves ion channels, so-called heat-sensitive transient receptor potential (TRP), as well as nitrite. TRP are calcium ion channels and the resulting increase in cellular calcium can activate several important signaling pathways and even activate transcription factors. Previous studies also postulate that FIR radiation is selectively absorbed by water molecules that are associated with TRP ion channels within the cell membranes [30]. Similarly, we observed that inorganic additives with FIR emissivity could induce the production of nitrite in muscle cells. The increase of nitrite amount could possibly result from the conversion of nitrate, as shown in the decreased amount of nitrate in the additive groups compared with the control group without additives. Nitrite is considered as a physiological storage pool of NO, nitric oxide is rapidly oxidized to nitrite and further oxidized via a slow and complex reaction to nitrate [31]. Furthermore, nitrite itself plays a role as

an important signaling molecule [32]. Nitrite was demonstrated to mediates several beneficial tissue responses, including mitochondrial protection by decreasing mitochondrial reactive oxygen species generation, increasing exercise efficiency by decreasing oxygen consumption and respiratory rate without impacting the energy production during exercise, as well as increasing mitochondrial number via PGC-1α pathway. In this study, we analyzed the mitochondrial biogenesis and found the increased mitochondrial copy number for additive 2 group and additive 3 group after 72 hours. Therefore, combining the previous study and our results, a possible pathway of FIR radiation might be proposed, FIR radiation could possibly induce the nitric oxide production, and further induce certain physiological effects, such as increasing mitochondrial biogenesis and energy expenditure.

5 Conclusions

In this study, fabrics with three different combinations of additives that possessed high infrared emission properties were developed. To assess their biological influence, we co-cultured the additive incorporated fabrics with human skeletal muscle cells to study the effects on the nitric oxide (NO) generation, a key signal molecule that involved in certain physiological pathways. We observed the higher amount of nitrite in cells, which were under the influence of fabrics with additives compared to one with the fabric without additive. Additive 3, where graphene was used as a doping agent, revealed the highest ability to increase the amount of nitrite in cells. Under its influence, the amount of nitrite level was 30-40% higher compared to the reference sample. As a physiological storage pool of NO, nitrite could be converted into NO and subsequently activates certain pathways, leading to the effects of vasodilation or increasing the energy expenditure. Furthermore, the increase of NO was not caused by inflammation, as the amount of inducible nitric oxide synthase (iNOS) was not significantly increased. The production of NO may be possible via activation of ion channels on the cell membrane. The increase of active NO may lead to other physiological pathways to influence the metabolism and energy expenditure. The increase in mitochondrial biogenesis could be a potential effect, which was observed as 1.23-1.25-fold change for samples with additives as compared to the reference sample. The observed effects on physiological biomarkers made it possible to develop functional fabrics using such additives by a convenient method, bringing more health benefits on the daily life and sport performance and recovery.

Author Contributions

Pengfei He: methodology, investigation, data curation, writing; Veerakumar Arumugam: fabric preparation, writing-review and editing; Aleksander Góra: additive and yarn preparation, analysis; Vitali Lipik: conceptualization, methodology, supervision, data curation, writing – review and editing. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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