Key Engineering Materials
ISSN: 1662-9795, Vol. 840, pp 324-329

Submitted: 2019-05-06
Revised: 2019-07-08
Accepted: 2019-11-11
Online: 2020-04-24

Comparison of Biocompatibility of Fibroin Cocoon *Bombyx mori* L., Mineral Trioxide Aggregate (MTA), and Resin Modified Glass Ionomer Cement (RMGIC) as Pulp Capping Materials to Human Primary Dental Pulp Cells

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Keywords: Biocompatibility, human primary dental pulp cells, MTT assay, fibroin, RMGIC (resin-modified glass ionomer cement), MTA (Mineral Trioxide Aggregate)

Abstract. One of the requirements of endodontic material is to have good biocompatibility of pulp tissue that has direct contact with the material, to promote the process of tissue repair. Reversible pulpitis needs direct pulp capping treatment with medicament materials i.e. RMGIC (Resin-Modified Glass Ionomer Cement) and MTA (Mineral Trioxide Aggregate) which are used as the gold standard for direct pulp capping treatment. Both of the materials have several disadvantages including the necrotic tissue in the area has direct contact with the pulp so that it affects the successful treatment results. Nowadays, the new materials are being developed which are expected to improve the existing material deficiencies. In this study, fibroin was extracted from *Bombyx mori* L. cocoon. This study aimed to examine the biocompatibility of fibroin as a new pulp capping material with RMGIC (Fuji II LC, GC, Japan) and MTA (Rootdent, Technodent, Russia). An experimental study was conducted using extracted human primary dental pulp cells *in vitro* through orthodontic treatment. A methyl thiazole tetrazolium (MTT) assay was employed to test biocompatibility using ELISA Reader 590 nm wavelengths for 24, 48, and 72 h, respectively. The findings showed that the biocompatibility of fibroin had the highest value of all. In conclusion, fibroin biocompatibility toward MTA and RMGIC as pulp capping materials can be aligned, however, there were no significant differences.

Introduction

A direct pulp-capping is a treatment for exposed vital pulp involving the placement of a dental material over the exposed area to facilitate formation of protective barrier and biological function [1]. The direct pulp-capping has been used as an alternative treatment to maintain vital pulp, thereby avoiding as many as 22 million root canal treatments annually [2]. The prognosis of direct pulp capping is lower than indirect pulp capping treatment. It was about 37% in 5 years and 13% after 10 years [3]. The finding indicates that the direct pulp capping treatment has a low success rate. Two pulp capping materials such as RMGIC and MTA have been widely used, however previous study have reported that RMGIC was not able to form tertiary dentin in human teeth and MTA have imperfect tertiary dentin [4].

Examining the biocompatibility of fibroin is absolutely necessary as it is a pulp capping material in the future to meet the need of current material [5]. Fibroin contains 18 amino acids and has been used for medical treatments; suturing material, bone graft, and cornea and artificial ligaments [6]. The efficacy of fibroin as an organic material has been proven biocompatible in tissues [6]. Although extensive research has been carried out on biocompatibility of fibroin, no single study exists which reported fibroin derived from *Bombyx mori* L. that is cultivated in Indonesia with other pulp capping material. the purpose of this study was to examine fibroin with the gold standard for existing pulp capping materials.

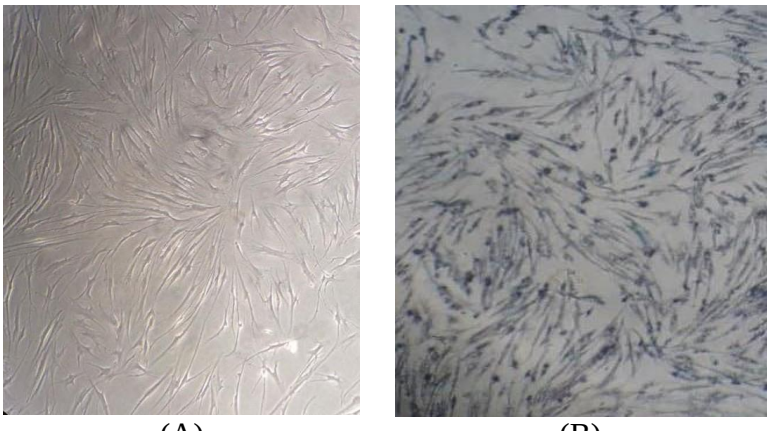
Experimental Section

Fibroin was extracted from *Bombyx mori* L. cocoon that was obtained from Silkworm Cultivation Centre, Central Java, Indonesia. An experimental study was conducted using human primary dental pulp cells *in vitro* for orthodontic purposes. The study has been approved by Medical Ethics of Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta, Indonesia under number 025/EP-FKIK-UMY/I/2019. The comparison of biocompatibility of fibroin, MTA (Rootdent, Technodent, Russia) and RMGIC (Fuji II LC, GC, Japan) was observed by evaluating human dental pulp cells viability employing MTT-assay (SIGMA-Aldrich, St. Louis,MO, USA) using 96-well plates with 7×10^3 seeded cells/well in 100 µL culture media. The cells were exposed to fibroin of *B. mori* L. cocoon, MTA and RMGIC. The concentrations of each material used were 100, 50, 25, 12.5, 6.25, 3.125, and 1.5625 µg mL^{-1} for 24, 48, and 72 h, respectively. Then the absorbance values were determined by ELISA reader with 595 nm wavelengths (Biotek, Winoosky, VT, United States). To count cells viability, the obtained-absorbances were normalized by the absorbance of the control cells (unstained cultured cells-as 100% viability condition) times 100% [7]. The percentage of cell viability was calculated using the formula as follows:

$$\text{cell viability}\,(\%) = \frac{\text{mean absorbance of well} - \text{tested}}{\text{mean absorbance of untreated well}} \text{x100}$$

Results and Discussion

The main pulp cells were odontoblast cells, fibroblast cells, undifferentiated ectomesenchymal cells, macrophages and immunocompetent cells [8]. Primary cells are cells obtained directly from the separation of tissue of an organ through cutting normal tissue and culturing (Sigma-Aldrich, 2009). Primary pulp fibroblast cells of human teeth cultured using DMEM media were seen using an inverted microscope (Fig. 1A) and description of human dental pulp fibroblast cells after MTT-Assay, which shows that living pulp cells are characterized by the activity of enzymes that reduce MTT to purple formazan in living mitochondria, thus giving a purple appearance (Fig. 1B).



(A) (B)

Figure 1. Primary human dental pulp cells before pulp capping material exposure (A) and cells after pulp capping materials exposure that had purple appearance indicate the living cells (B)

For further study related to the biocompatibility of fibroin, RMGIC and MTA we utilized a yellow water-soluble tetrazolium dye (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) to be reduced enzymatically by mitochondrial dehydrogenases in a living cell to form formazan crystals which could be detected spectrophotometrically at 590 nm. The result of the viability test shown in Fig. 2, 3, and 4 showed that the primary human dental pulp cells viability was above 100% including the fibroin [9]. Based on ANOVA statistical calculations there were significant differences between fibroin, MTA and RMGIC, however, based on the laboratory observation the results of optical density by ELISA 590 nm reader showed that there were no differences among all materials and concentration because there was no significant difference between optical density in the cell control (unstained cell). There was an increase in the cell viability percentage unaffected by concentration.

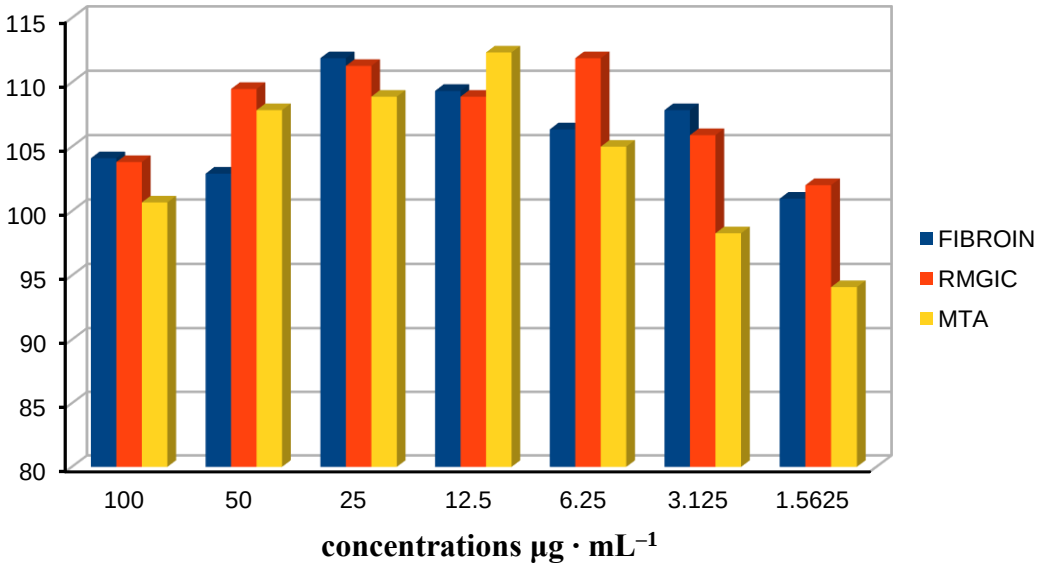


Figure 2. The viability of primary human dental pulp cells of *B. mori* L. fibroin, resin-modified glass ionomer cement (RMGIC) and Mineral Trioxide Aggregate (MTA) for 24 h MTT assay

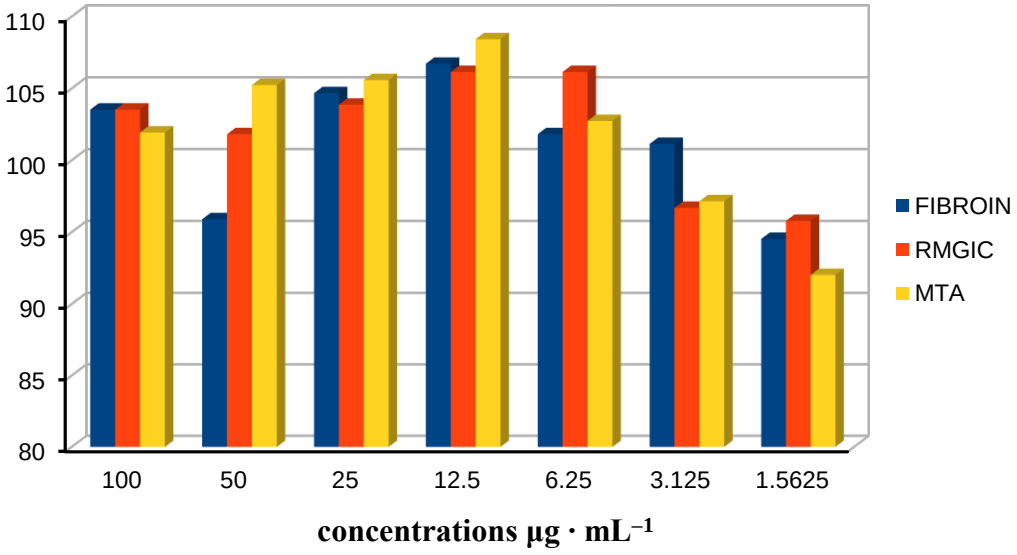


Figure 3. The viability of primary human dental pulp cells of *B. mori* L. fibroin, resin-modified glass ionomer cement (RMGIC) and Mineral Trioxide Aggregate (MTA) for 48 h MTT assay

Cell culture is an effort to grow in an artificial environment that is conducive to growth. Cell culture is divided into a primary and secondary cell (cell line). Primary cells were obtained directly from the separation of tissue of an organ through cutting normal tissue and culturing, in this study researcher used the pulp of the teeth (Sigma-Aldrich, 2009). Primary cultures can only be maintained within a certain period, while cell lines are cell offspring obtained from primary cell cultures and have been separated enzymatically or mechanically. Cell culture media contains DMEM, carbohydrates, serum, and antibiotics [10].

Biocompatibility is defined in the Dorland's Illustrated Medical Dictionary as harmony with life and does not have toxic effects or detrimental effects on biological functions especially in the oral cavity. Based on ANSI/ADA Document number 41 (1979) biocompatibility examinations of dental materials are divided into three examinations; initial examination including in vitro examination, secondary examinations including in vivo examination, in small animals, and use tests include in vivo examination in larger animals or humans. In vitro examination can be done through several types of examinations including cytotoxicity tests which measure the toxic effects of materials on cells [11].

Cytotoxicity tests can be carried out using both primary and secondary cell types. In this study, the use of primary cell cultures is more relevant in assessing the cytotoxicity of a material compared to the cell line because primary cells are not transformed so that cell characteristics remain the same. The two common methods used for cytotoxicity test are the dye exclusion test and colorimetric test using the MTT assay. Colorimetric test method using MTT assay or MTT assay test [3- (4,5-dimetyltiazol-2-yl)-2,5-diphenyltetrazolium bromide] is one of the cytotoxicity test methods based on the activity of living cell mitochondrial reductase enzymes that reduce compounds of methyl thiazole tetrazolium (MTT). The MTT test is very sensitive for evaluating cell viability. MTT assay is a standard laboratory and colorimetric test (based on color changes) to measure the activity of enzymes that reduce MTT to purple formazan in living mitochondria, thus giving a purple appearance (Fig. 1B). The reaction can be used as an index of cell viability. Dead cells will not have this reaction because they do not have mitochondrial dehydrogenase enzymes [12].

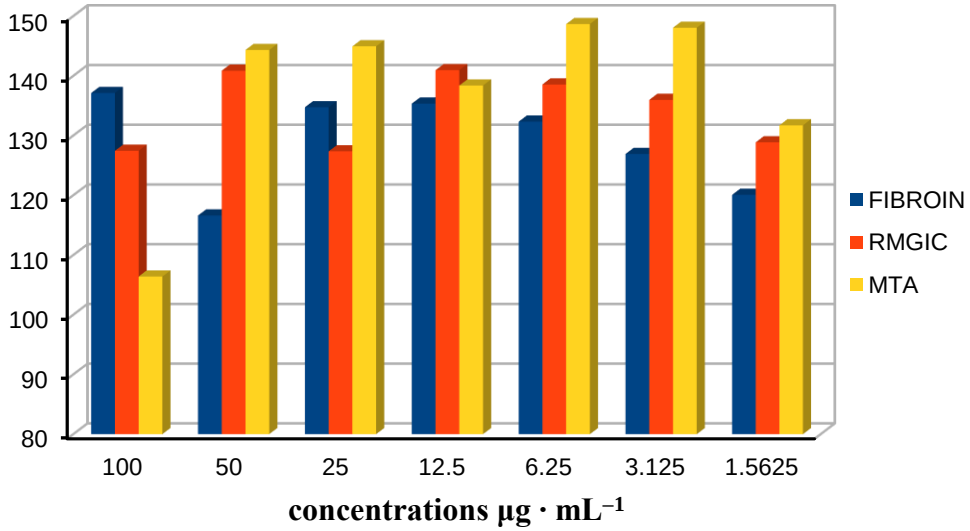


Figure 4. The viability of primary human dental pulp cells of *B. mori* L. fibroin, resin-modified glass ionomer cement (RMGIC) and Mineral Trioxide Aggregate (MTA) for 72 h MTT assay

The basic principle of MTT assay is to measure cellular activity based on mitochondrial succinate dehydrogenase activity to reduce MTT salts. In the process of metabolism, living cells produce succinate mitochondrial dehydrogenase. The enzyme reacts with MTT salts and forms purple formazan crystals whose numbers are comparable to living cell activity [13]. The absorbance value (OD) of formazan crystals that have been dissolved can be measured using a spectrophotometer (Elisa reader and microplate reader) with a specific wavelength. The purple formazan concentration can be determined by visible spectrophotometry and is directly proportional to the number of living cells

because reduction only occurs when the reductase enzyme contained in the cell respiration pathway in active mitochondria. When the certain amount of purple formazan is produced by treated cells compared to control cells that do not get treatment, the effectiveness of the agent that causes purple formazan dissolves in water can be dissolved in the reagent stopper to stop the MTT reaction with sodium dodecyl sulfate, or DMSO, or isopropanol acidified. Viability is stated by comparing the absorbance value of the treatment group exposed to the tested material with the control group (samples without tested material). The greater absorbance indicates the more the number of living cells [13, 14].

The MTT test was carried out for biocompatibility analysis and the results showed no association with higher concentration, the higher cell viability. As seen on Fig. 2, 3, and 4 the cytoviability of pulp cells at 24 and 48 h was above 90% after exposure of the materials while at 72 h, cytoviability of the pulp cells was above 100%. However, the high viability of the cells does not indicate that the material can promote the proliferation of primary human pulp cells because the concentration of the material did not affect the viability of primary human pulp cells.

In this study, fibroin as a pulp capping material has good biocompatibility with human dental pulp cells because it has high cell viability (up to 90% in cell survival), which is the same as the gold standard pulp capping material. Therefore, the fibroin is safe to use as a direct pulping material because it does not cause cytotoxicity.

Summary

According to our findings, it can be concluded that fibroin biocompatibility toward MTA and RMGIC as pulp capping materials can be aligned and there is no significant difference.

Acknowledgment

The authors would like to thank Universitas Gadjah Mada's Publishers and Publications Board Yogyakarta Indonesia for financial support with the grant number 2127/UNI/DITLIT/DIT-LIT/LT/2019.

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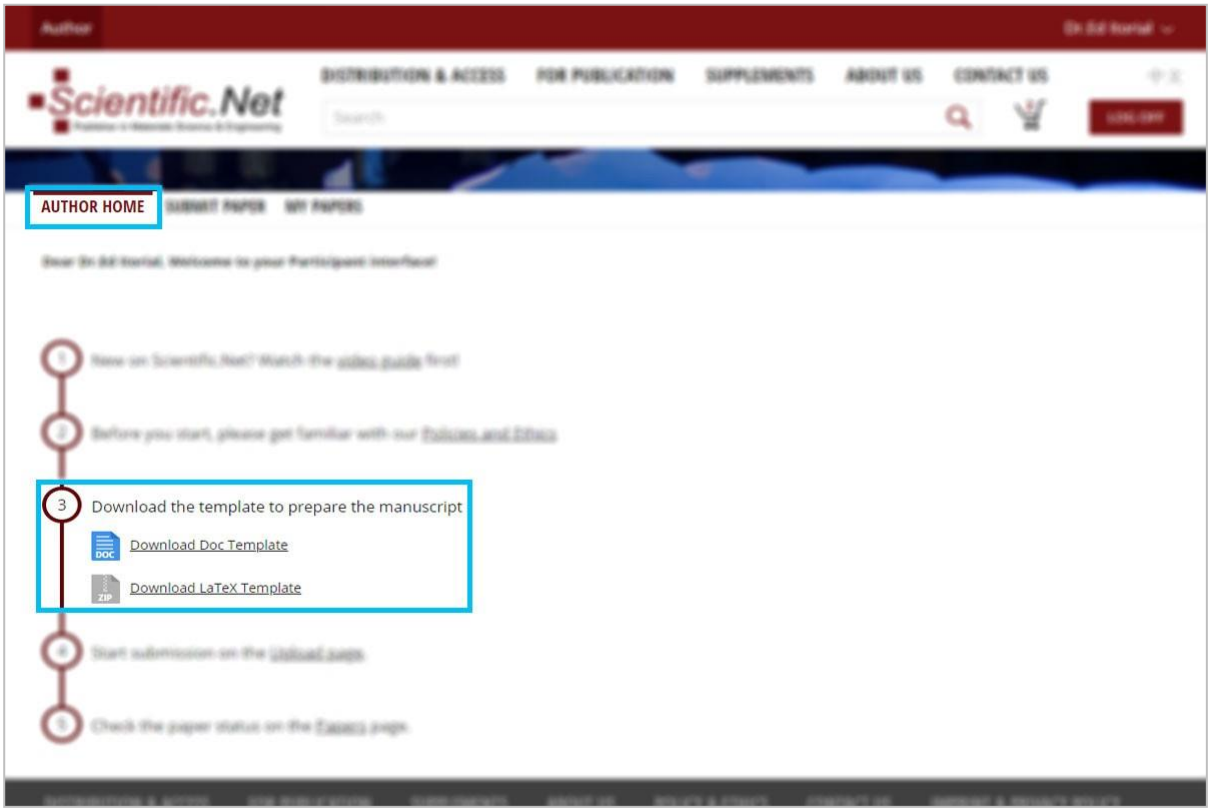

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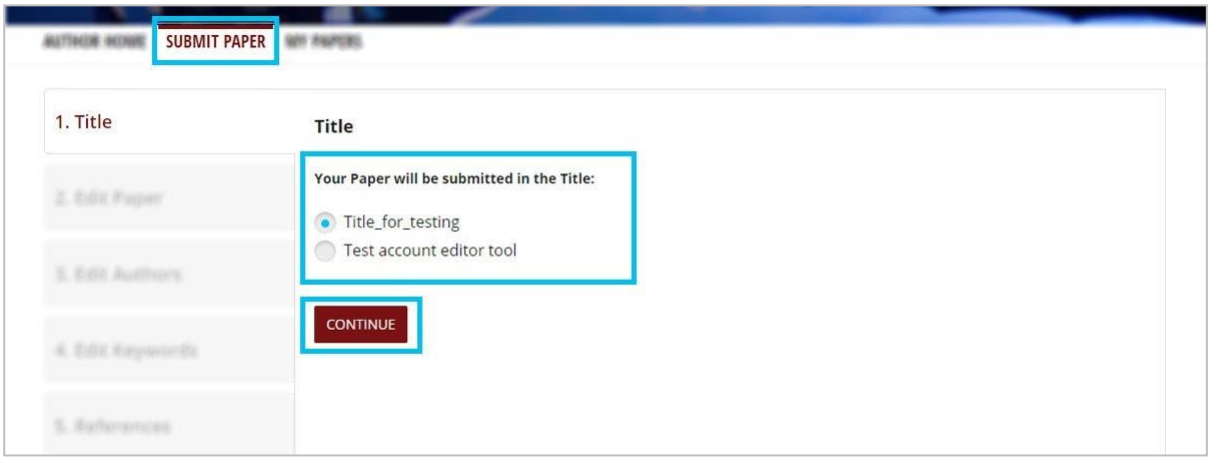

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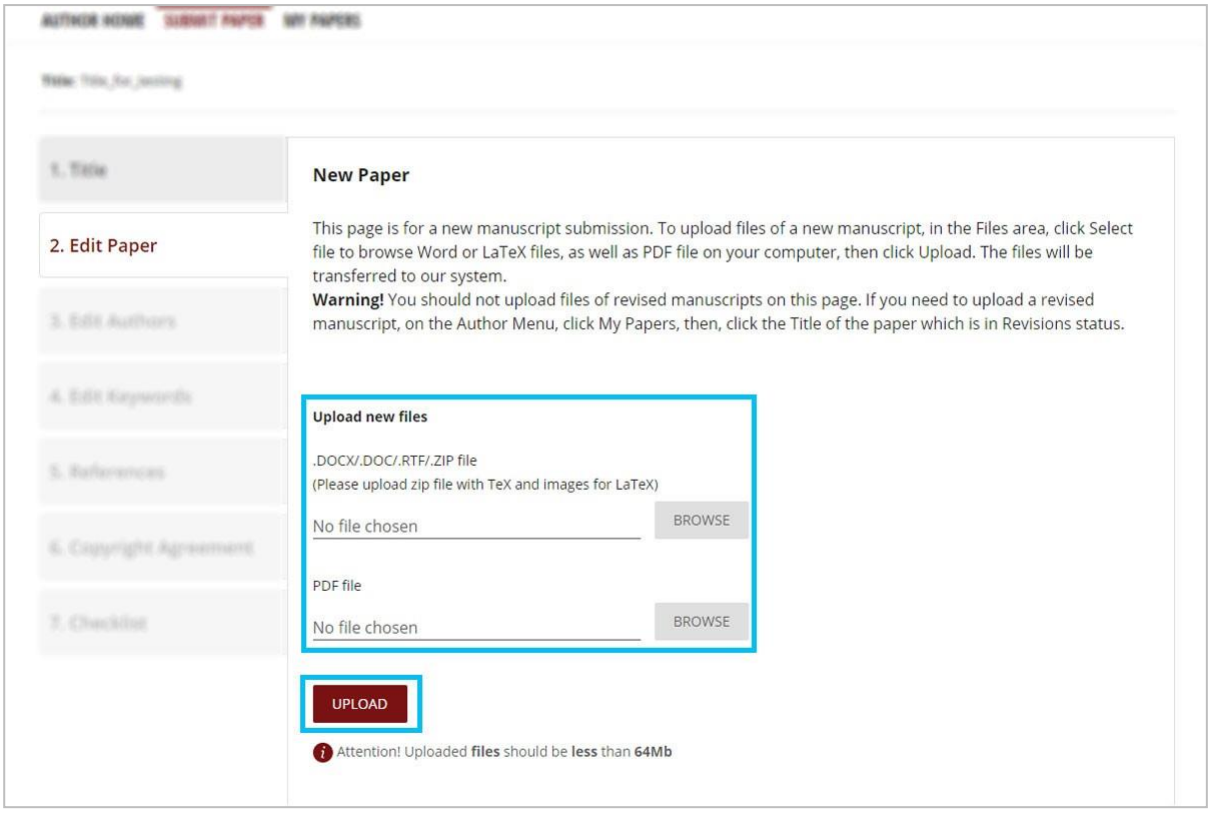

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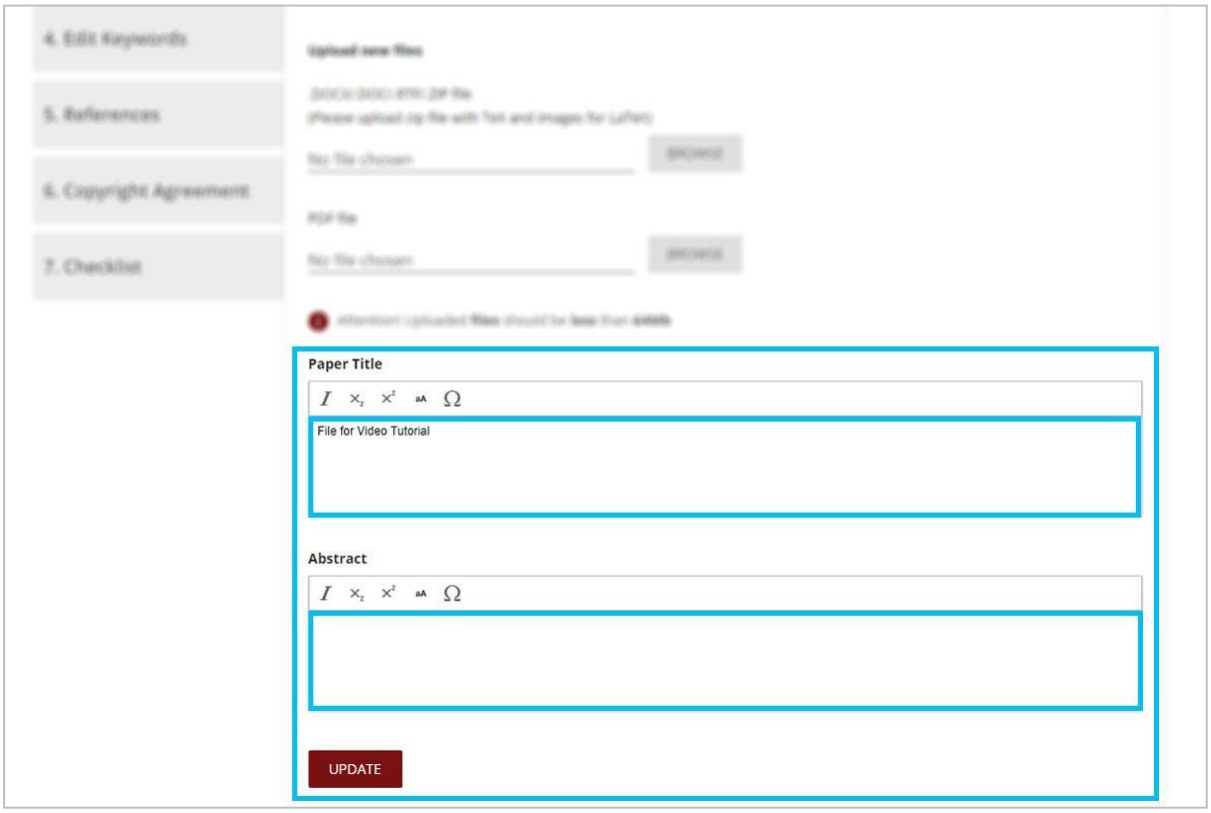

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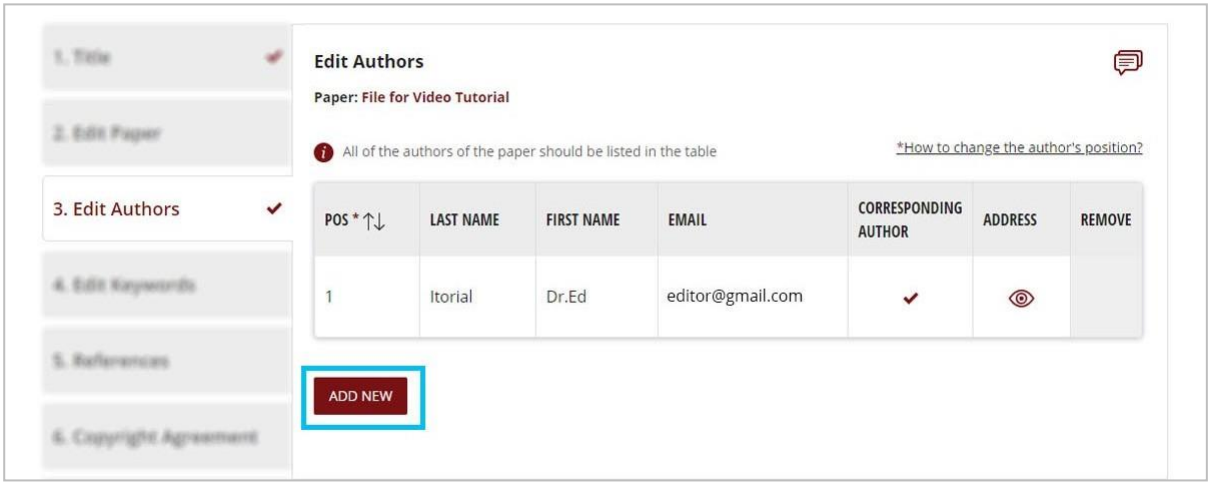

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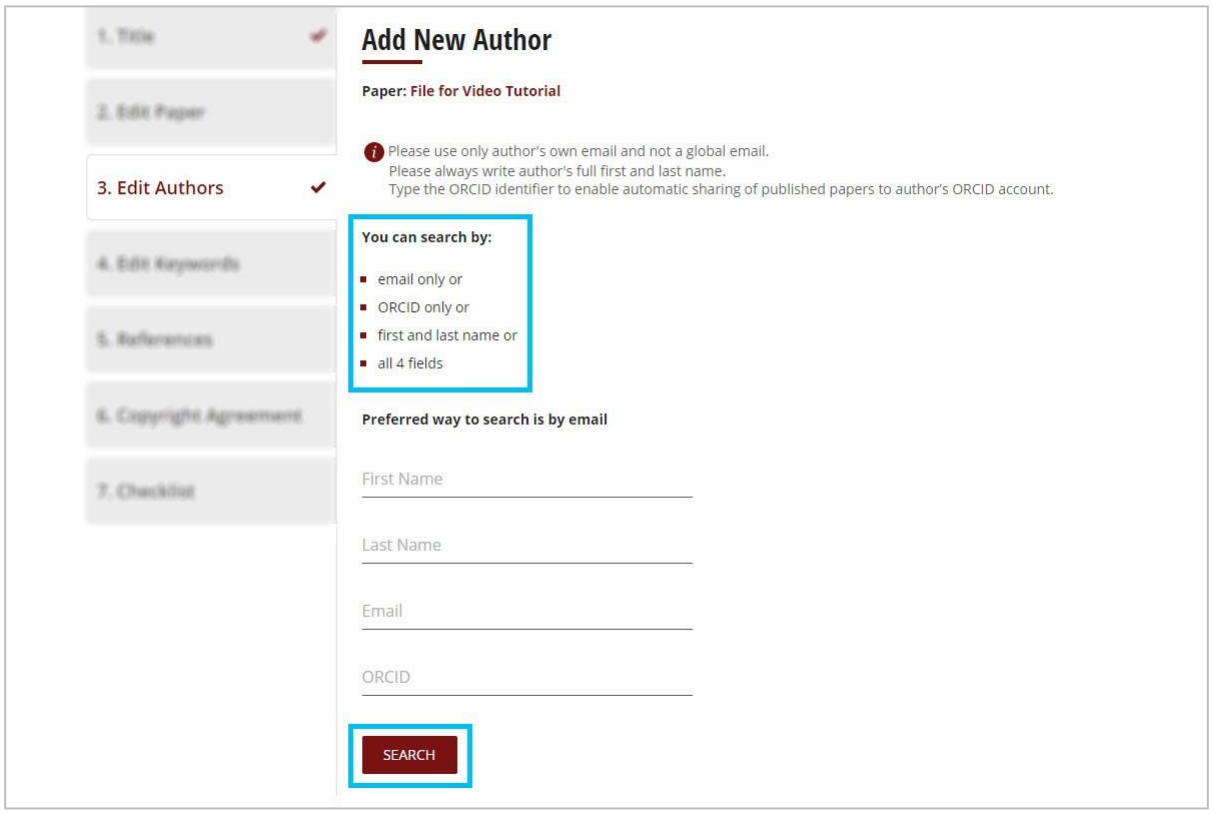

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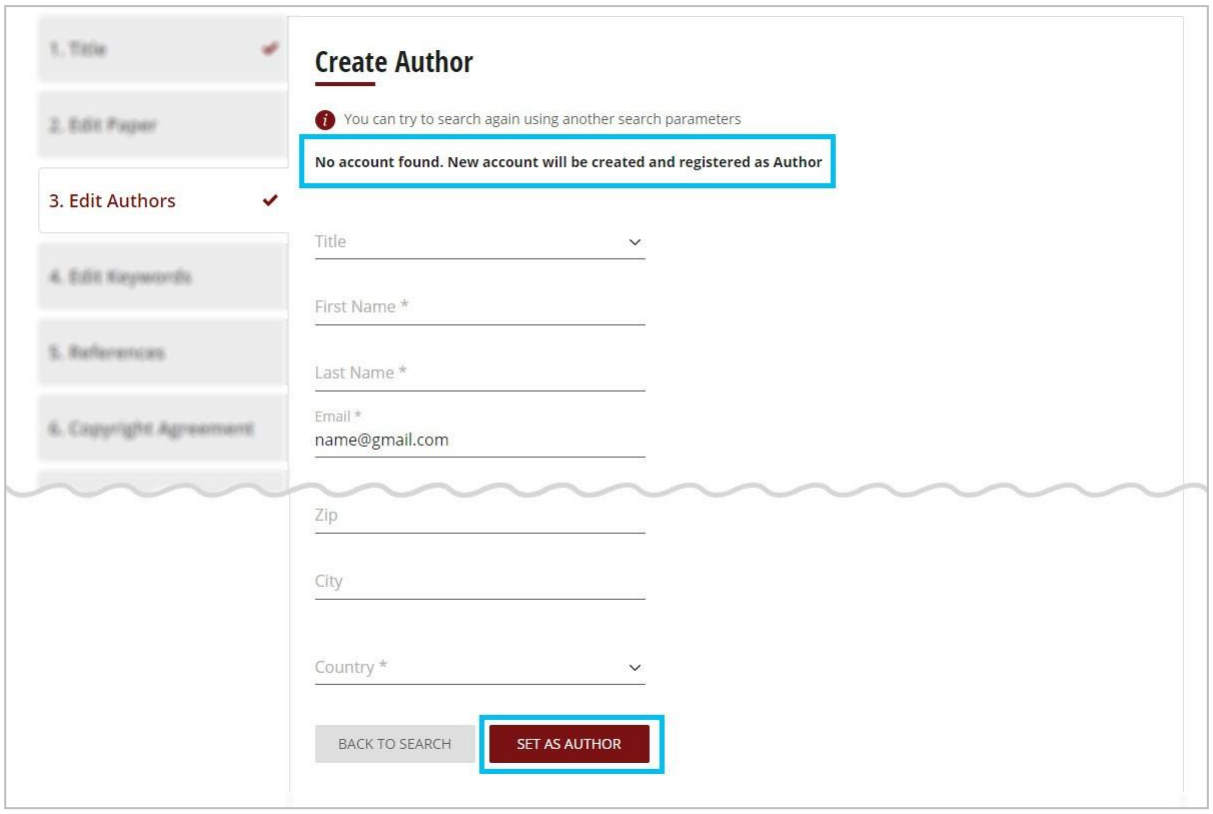

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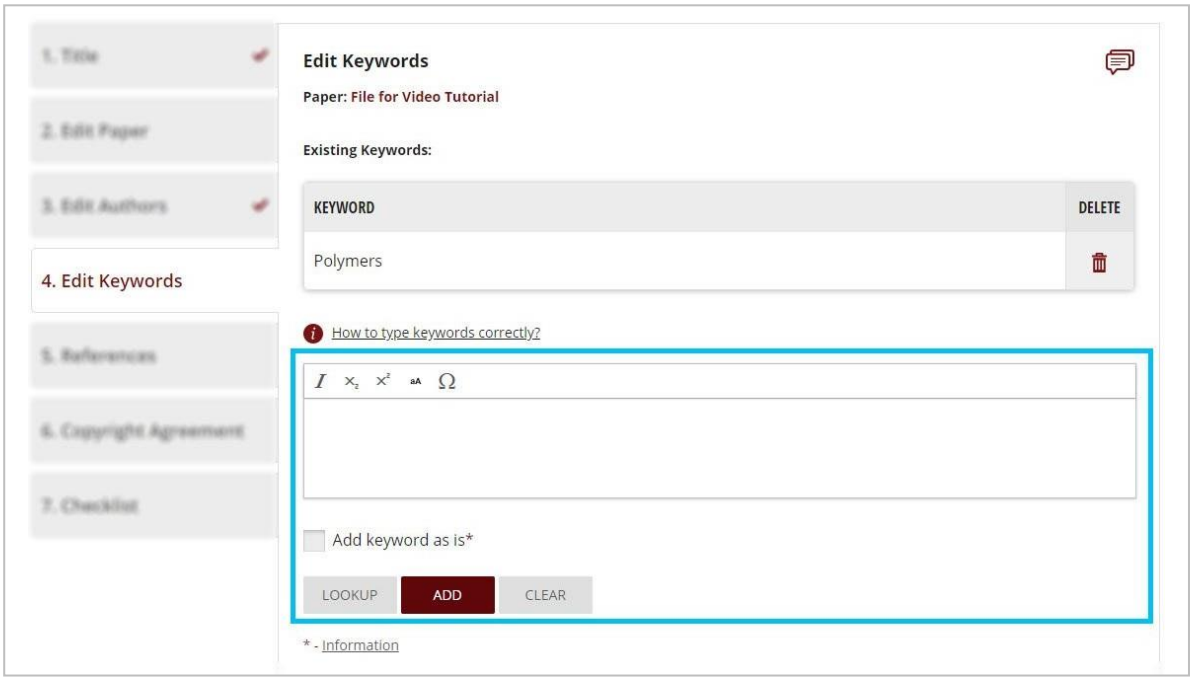

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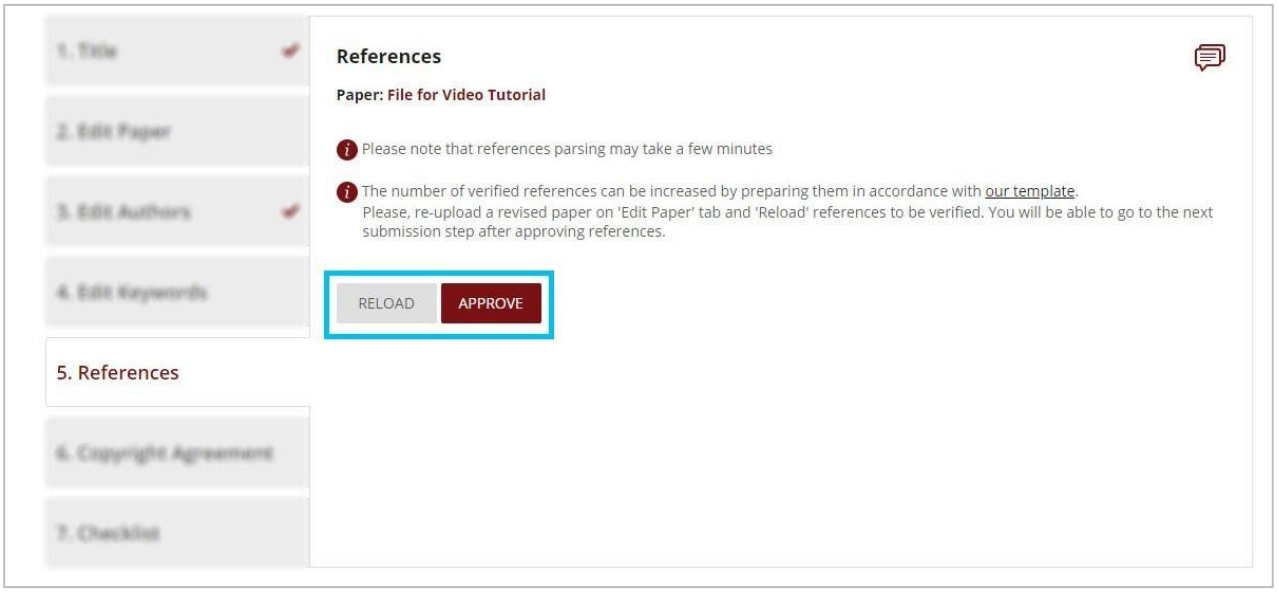

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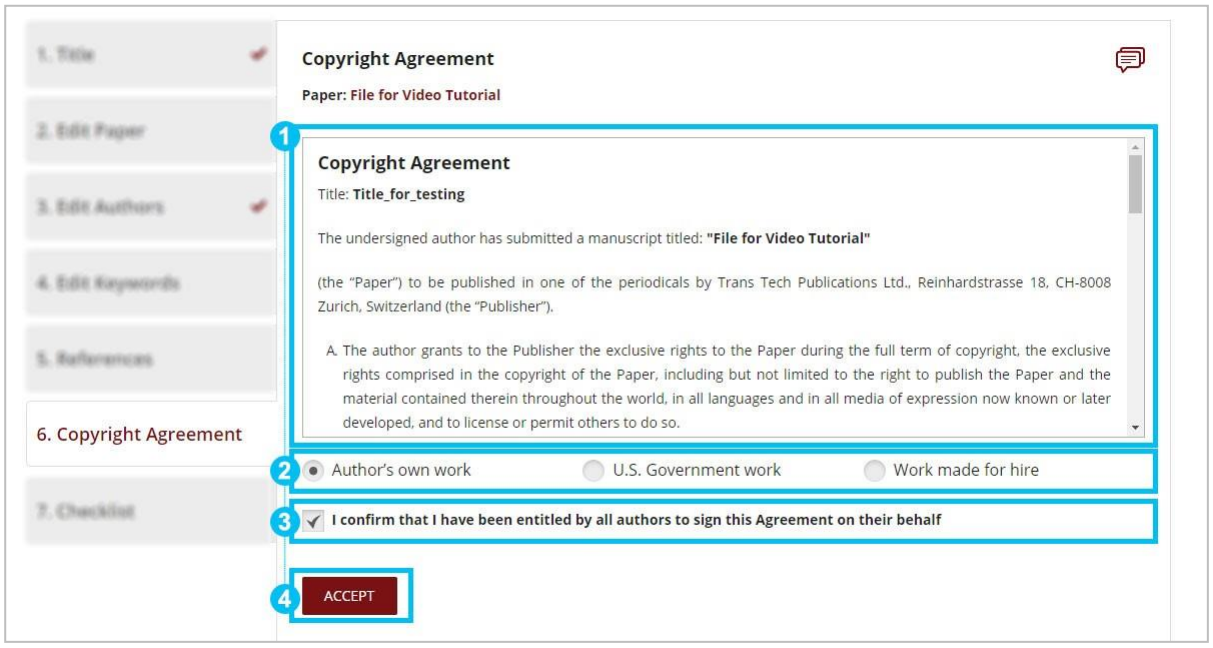

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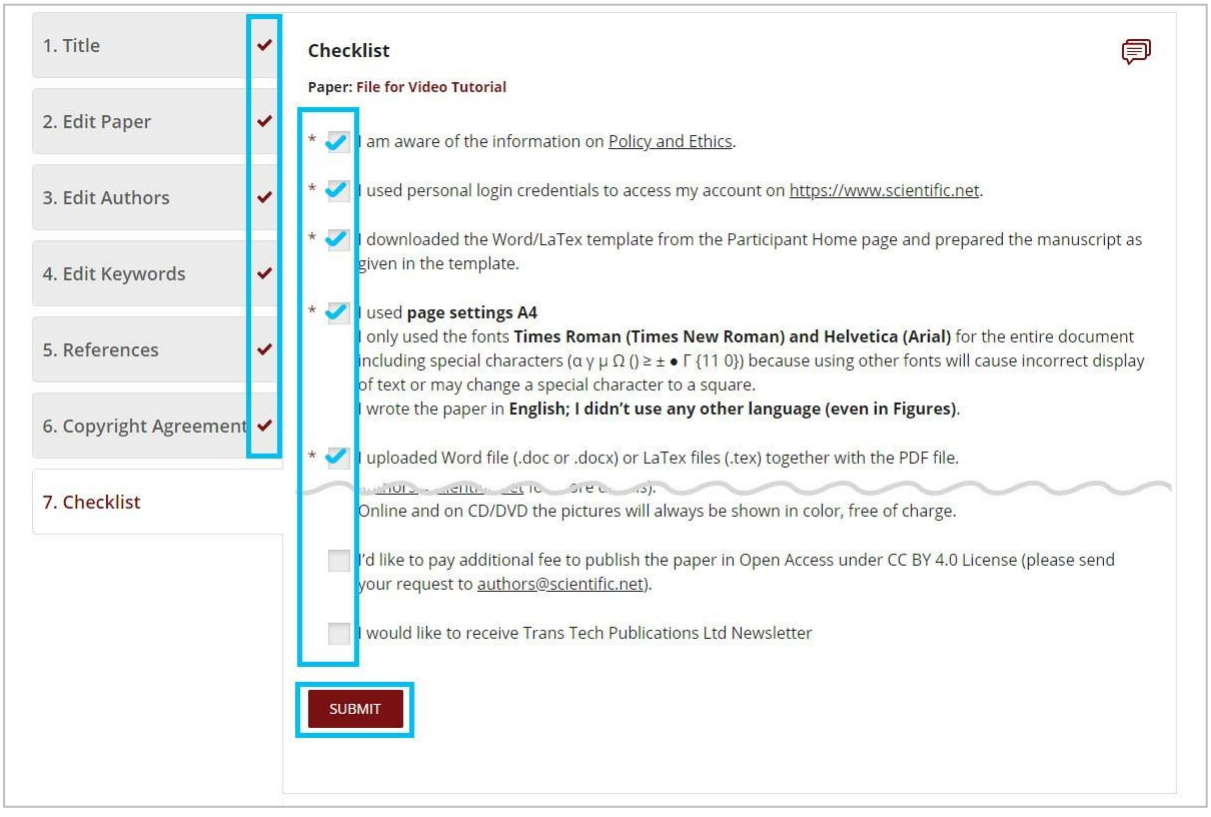

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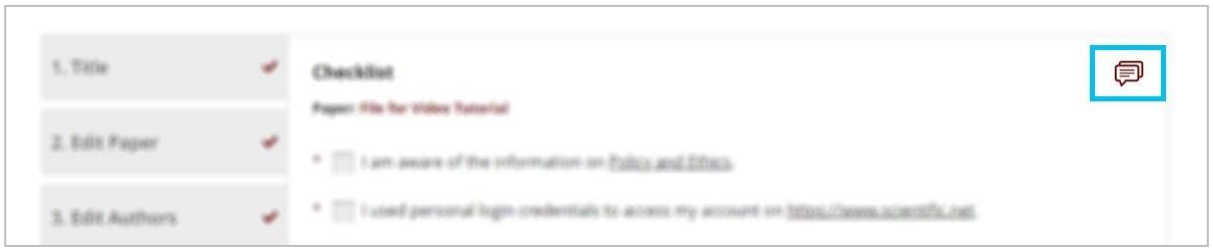

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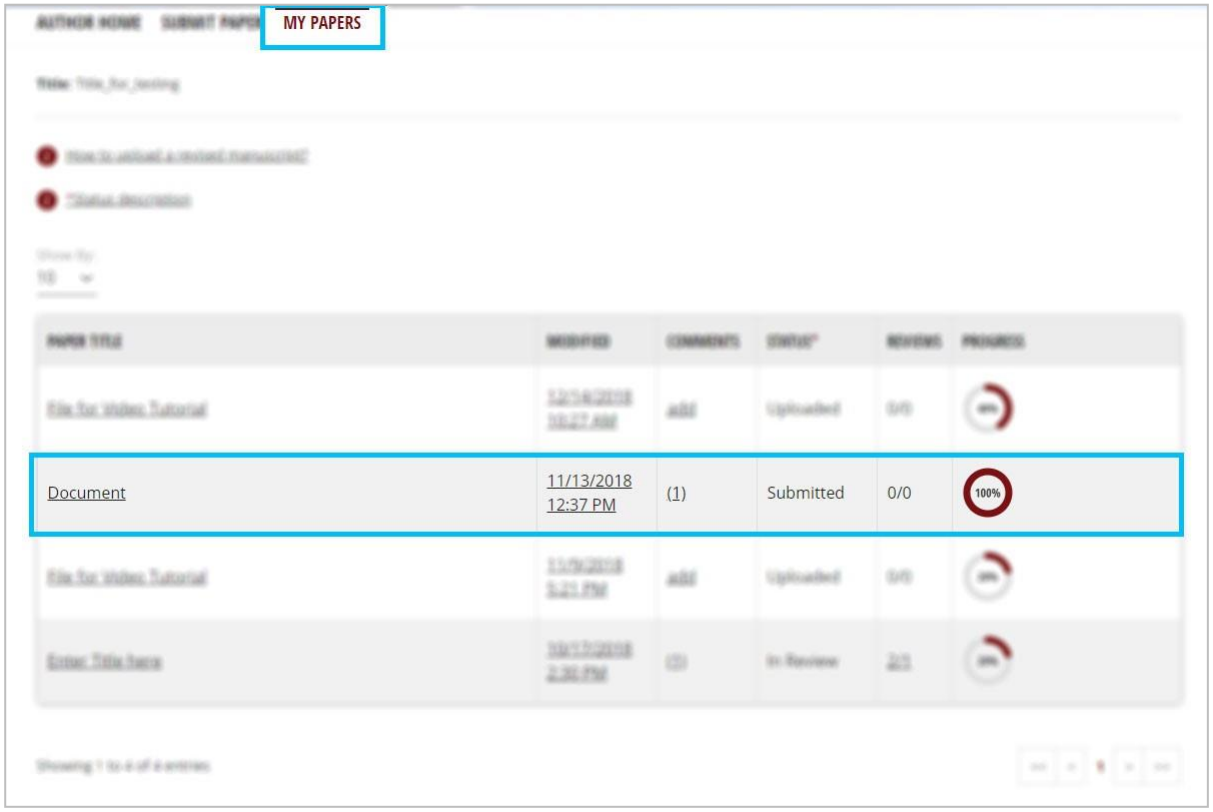

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