ESTABLISHING A 3D BIOPRINTED VASCULARIZED CO-CULTURE LUNG TISSUE MODEL USING LUNG FIBROBLASTS & ENDOTHELIAL CELLS

Juan Brussow[1,2], Sakshi Phogat[1,2], Fama Thiam[1,2], Emmanuel T. Osei[1,2]

[1]University of British Columbia – Okanagan Campus, Biology Department [2]University of British Columbia – Okanagan Campus, Lung Bioartificial Model Lab,

LUNG BIOARTIFICIAL MODEL LAB. (OSEI LAB.)

INTRODUCTION

Traditional animal & 2D cell culture models present a challenge for the accurate study of chronic lung disorders such as lung cancer, asthma, & COPD. This is because they fail to replicate the complex tissue structure & vasculature of the *in vivo* human lungs, hindering biomedical research translation. Extrusion-based bioprinting offers a solution via the creation of multicellular 3D *in vitro* models overcoming these limitations, and enabling the study of complex cellular communication. In line with this, the BIOX extrusion-based bioprinter by CELLINK® is one of the few bioprinters with carefully optimized and standardized protocols that is widely used & trusted in the field. Like all extrusion-based bioprinting it enables the creation of tangible 3D engineered models via the use of pressure, delivered through a syringe/nozzle to dispense cell-seeded bioink or hydrogel mixtures. Hydrogels which make up most bioinks are cross-linkable polymer networks that form 3D structures to mimic the ECM of different tissues including the lungs. Together with the BIOX & commercially available devices such as the Vaskit® that enable channel creation in established models, complex models mimicking the vasculature in the mesenchyme can be established. An engineered vascularised model of cell-seeded lung ECM offers a platform for further studies of complex cell function & crosstalk in health & disease.

AIM

The aim of this study was to create an optimized protocol to establish a bioprinted vascularized 3D *in vitro* co-culture model that mimics the fibroblast embedded subepithelial space of the lung

MATERIALS & METHODS

- 3D models were bioprinted using the Vaskit® mold, the BIO X Bioprinter (CELLINK®), Gelatin methacryloyl (GELMA) and sacrificial Pluronics hydrogel bioinks, MRC-5 lung fibroblasts and the EaHY926 endothelial hybrid cell-lines.
- MRC-5 lung fibroblasts were mixed with liquid GELMA, and photocrosslinked in Vaskit® using the BIOX
- A channel of a specific size and shape that aligns with the inlet(s) and outlet(s) of the Vaskit was designed, uploaded and carefully bioprinted with Pluronics
- Bioprinting was carefully done so the sacrificial Pluronics was on top of the already photopolymerized GELMA layer and it aligned with the inlet and outlet inside the Vaskit.
- A mixture of MRC-5 fibroblast-GELMA mixture was added on top of the bioprinted sacrificial Pluronics and also photocrosslinked with the BIOX.
- The sacrificial Pluronics was then washed away to form patterned lumens in the GELMA model which is continuous with the inlet and outlet of the Vaskit
- Basement membrane proteins were used to coat the created lumen before seeding endothelial cells in the channel to fill it.
- The model was then rotated using an automated rotator to facilitate the attachment of the endothelial cells before the model was connected to a syringe pump for culture media perfusion
- The engineered model was then perfused at a low flowrate of 5μL/min for 48 hrs, then 30-90μL/min for a week
- After experiments, the bioprinted models were immunostained with Phalloidin for F-actin (Green), E-cadherin for endothelial cell attachment and DAPI for all nuclei before confocal imaging was done

Vaskit Perfusion device

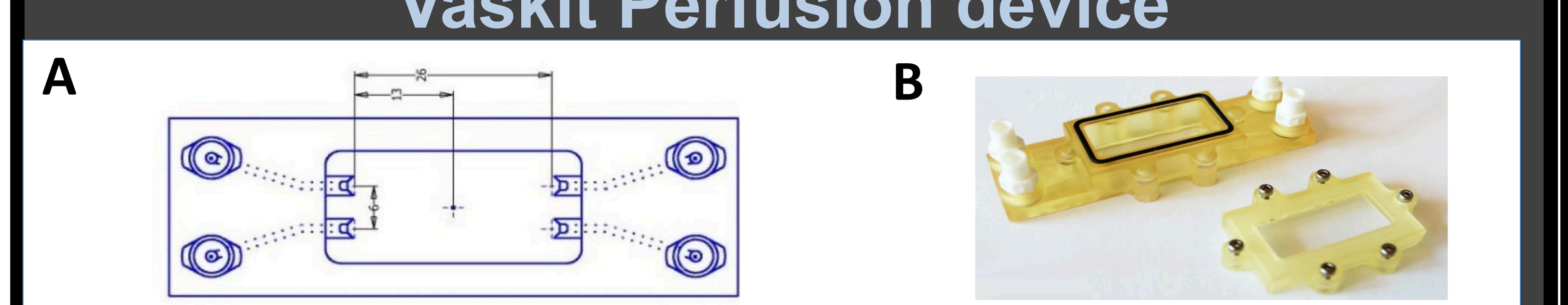

Fig 1.Vaskit device. A) Blueprint of CELLINK microvasculature Vaskit® perfusion device. B) Vaskit® with lid attachment seal for sterile 3D cell culture.

Extrusion Bioprinter & Vascularized 3D model set-up

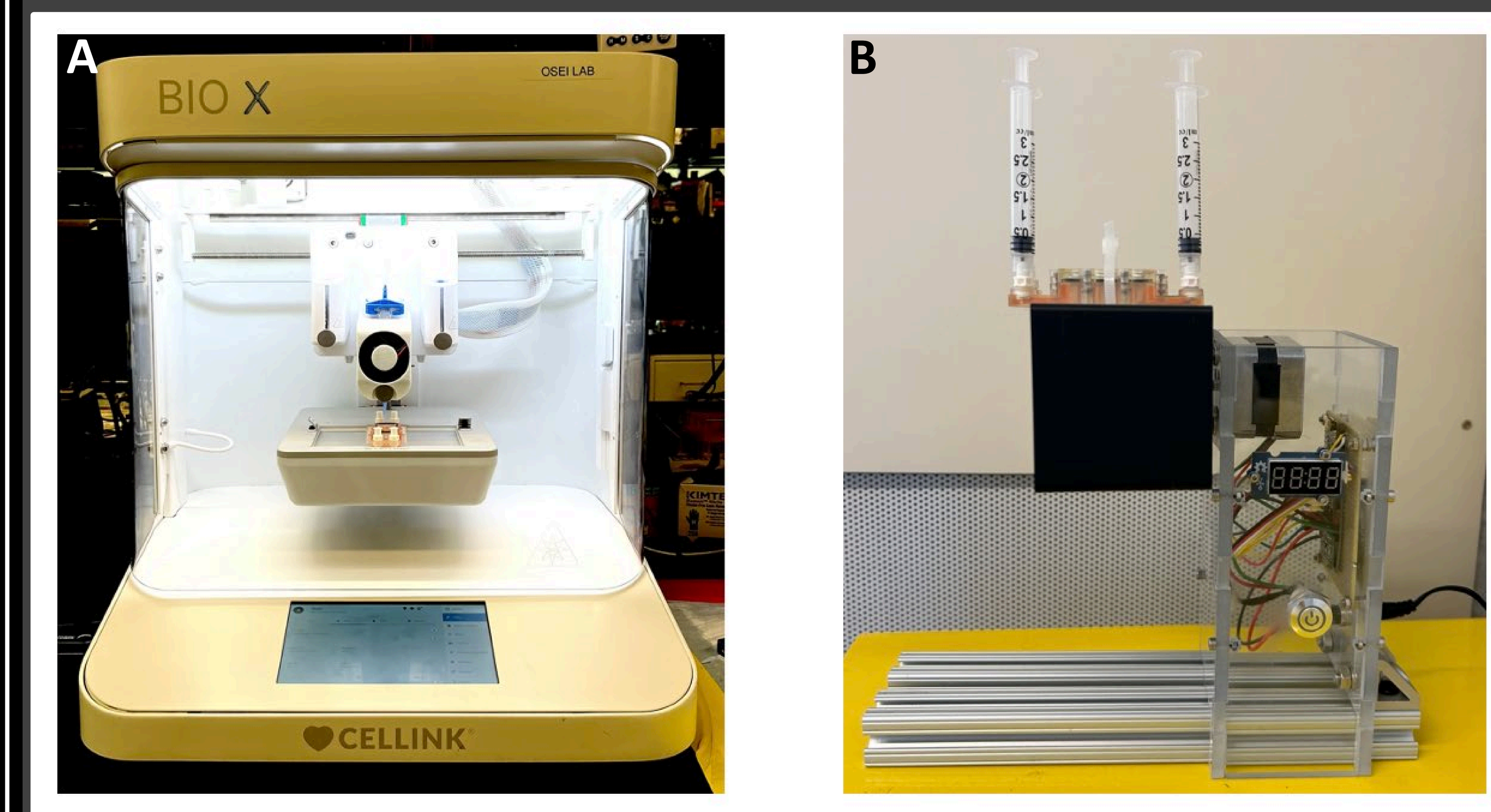

Fig 2. Experimental setup. A) BIO-X 3D Bioprinter with vaskit® device. B) Gel Flipper Device used for channel coating.

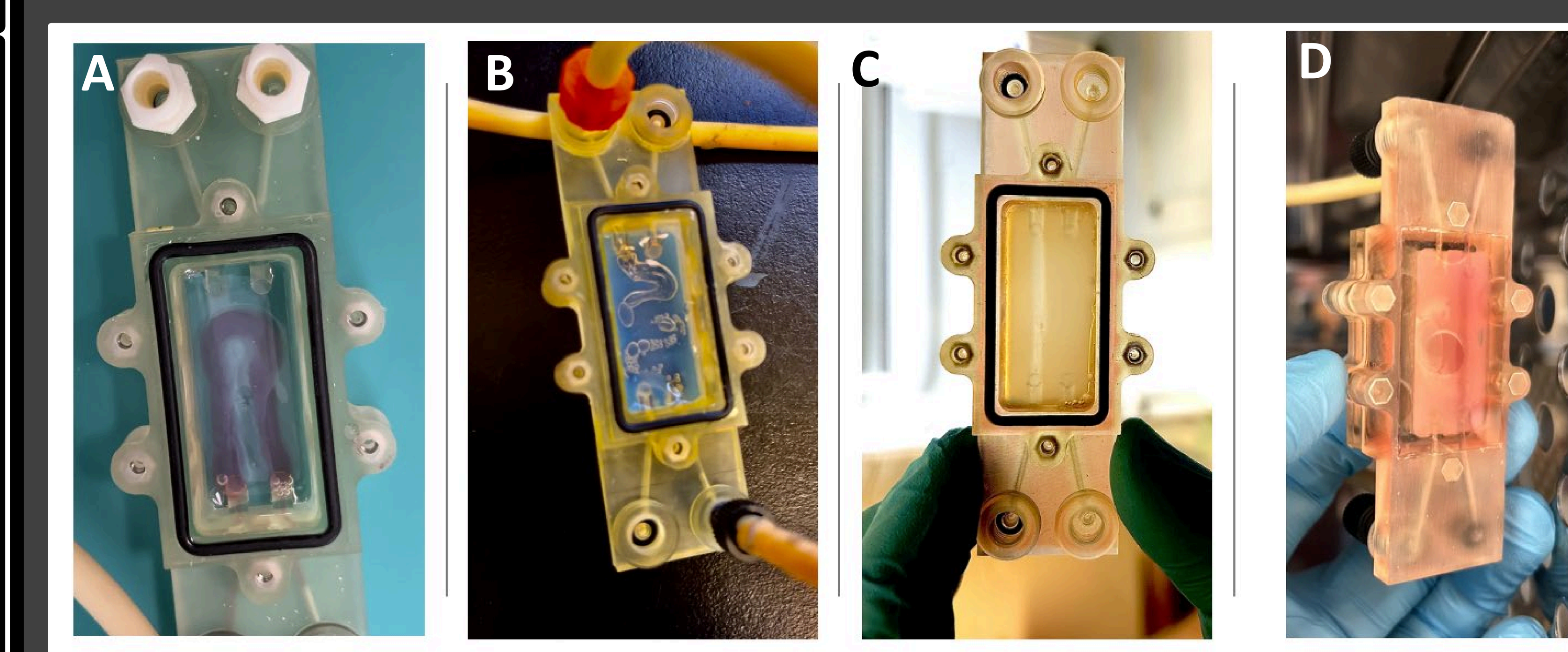

Fig 3. 3D Bioprintred Model Development Original printed model with A) looped & B) S -shaped channel, C) Final printed model with straight optimized channel. D) Final printed model using straight optimized channel showing media flow.

Figure 4. Microfluidic flow set-up . A) Original Peristaltic Pump Set-up showing pump with simple media bottle and tube. B) Optimized Syringe Pump Set-up showing holder for media bottle connected to Vaskit® device with cell-embedded bioprinted model

RESULTS

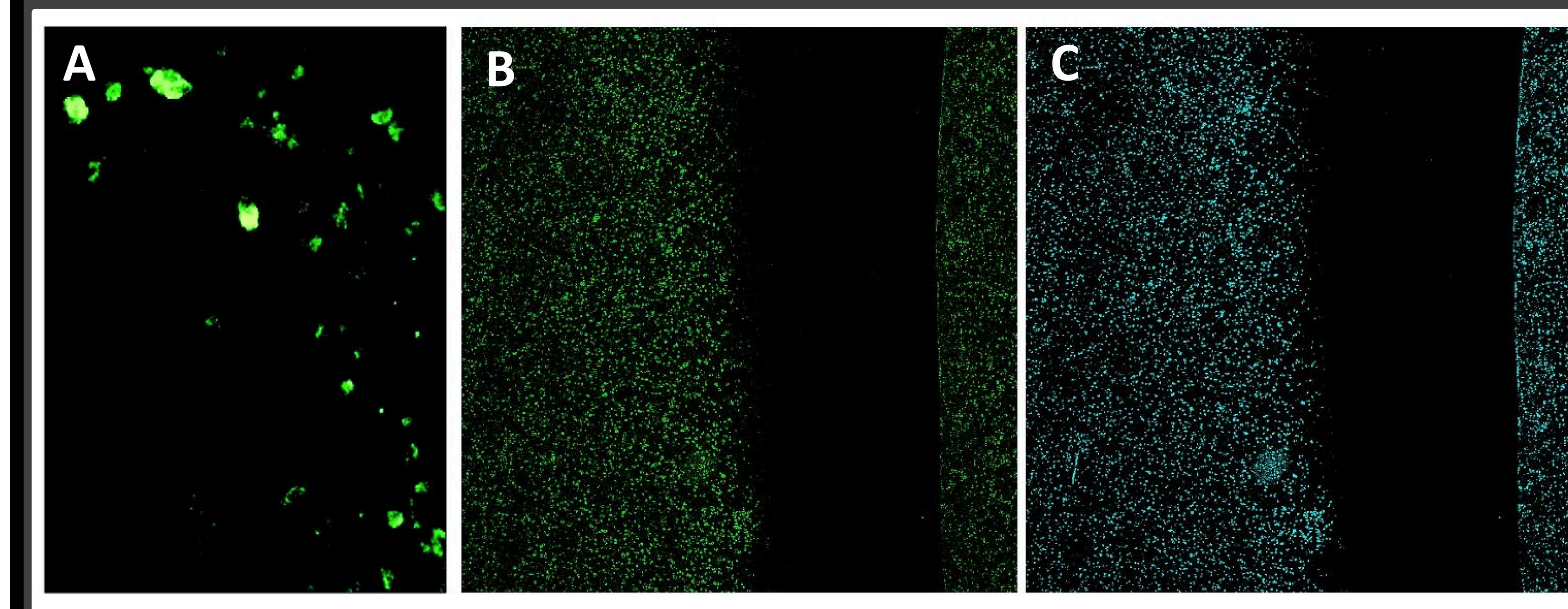

Figure 5. Immunostaining of Lung Fibroblasts-embedded in hydrogel surrounding vascularized channel in models. A) F-actin stained lung fibroblasts in trial model. B) F-actin & C) DAPI -stained lung fibroblasts showing successfully embedded cells in bioprinted GELMA model

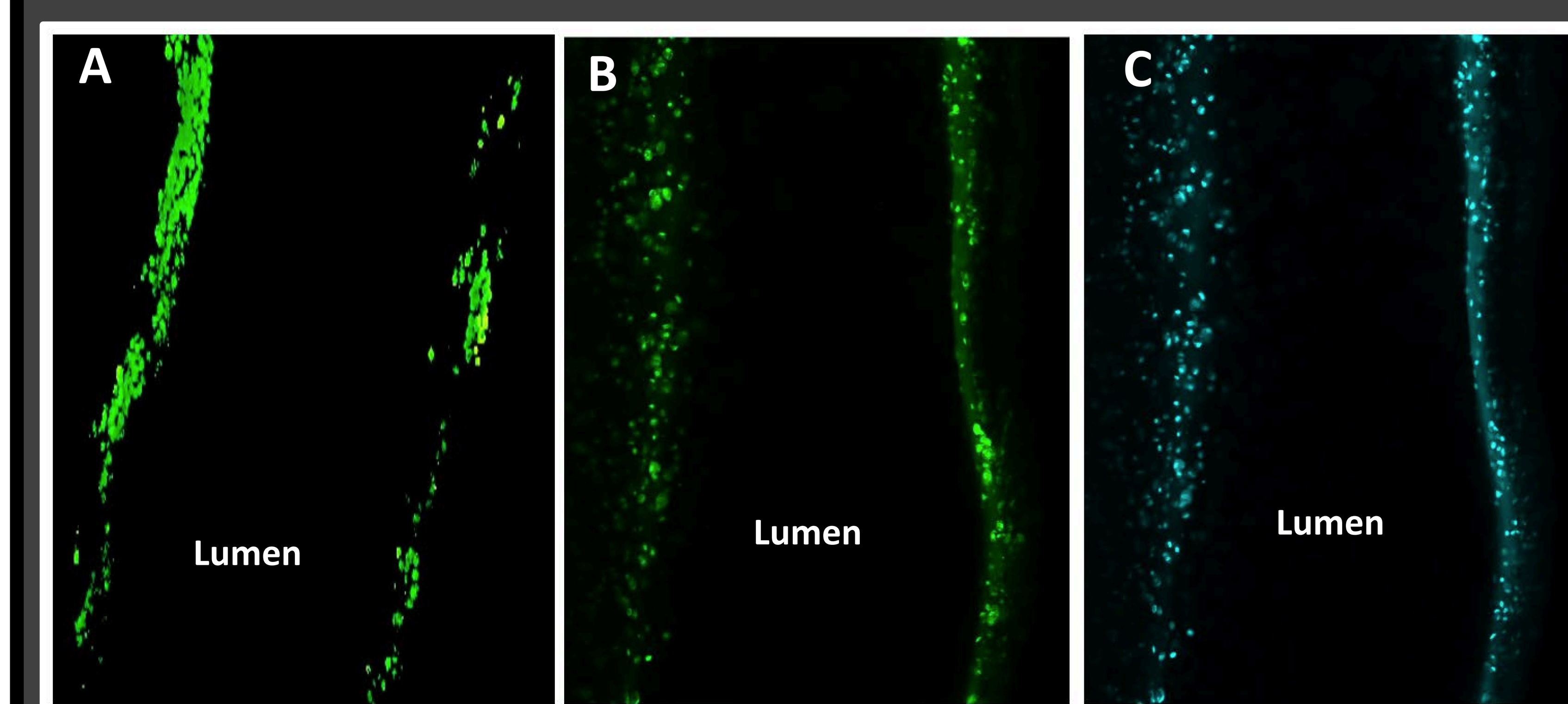

Figure 6. Immunostaining of Endothelial cells lining the vascularized channel in models. A) F-actin stained Endothelial cells in trial mode B) F-actin & C) DAPI -stained endothelial cells showing vascularization in bioprinted GELMA model

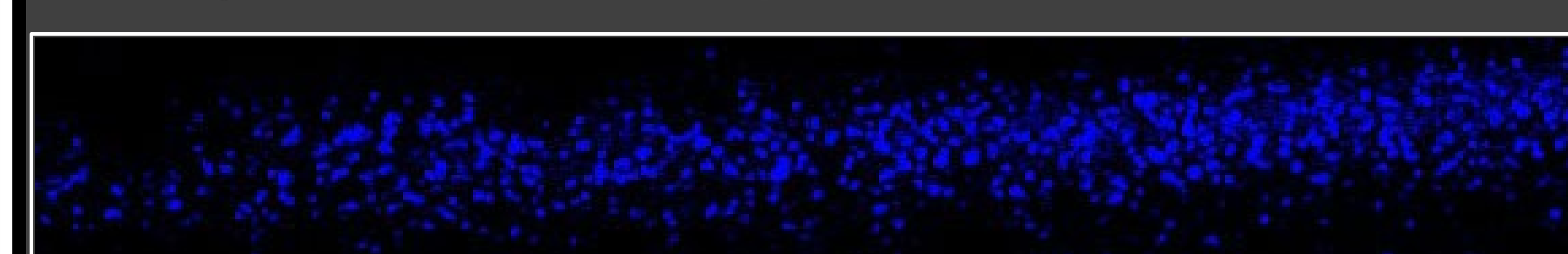

Fig 7. Apical view of Immunostaining of Endothelial Cells lining channel. E cadherin immuno-stained lung endothelial cells seeded in vascularized lumen shown apically.

CONCLUSIONS

- This project successfully established an optimized bioprinting protocol for engineering a 3D lung fibroblast-endothelial co-culture model with the BIOX extrusion based bioprinter.
- As a mimic of the vascularized subepithelial space in the lungs, the established model has potential for future studies into complex mechanisms in lung diseases (e.g., asthma, COPD).

THE UNIVERSITY OF BRITISH COLUMBIA

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References