Effects of Simulated COVID-19 Cytokine Storm on Stent Thrombogenicity

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ABSTRACT

Background: Cytokine storm-related hypercoagulation may be important in the pathogenesis of stent thrombosis in patients with SARS-CoV-2. Whether stent polymers behave differently under such conditions has never been explored.

Methods: Fluorinated polymer-nanocoated and uncoated COBRA stents (CeloNova), BioLinx-polymer-coated Resolute Onyx stents (Medtronic), and Synergy stents (Boston Scientific), which are abluminally coated with a bioabsorbable polymer, were exposed to human blood from healthy donors which was supplemented with 400 pg/mL IL-6 and 100 pg/mL TNF-α, similar to what is seen in cytokine storm caused by SARS-CoV-2. Platelet adhesion and neutrophil activation, assessed by immunofluorescence, were compared under cytokine storm and control conditions (untreated blood) ($n = 4$ experimental runs).

Results: Platelet adhesion values, defined as %platelet-covered area x staining intensity, were significantly lower in coated and uncoated COBRA and in Resolute Onyx than in Synergy under control conditions ($1.28 \times 10^7 \pm 0.43 \times 10^7$ vs. $2.92 \times 10^7 \pm 0.49 \times 10^7$ vs. $3.57 \times 10^7 \pm 0.73 \times 10^7$ vs. $9.94 \times 10^7 \pm 0.99 \times 10^7$; $p \leq 0.0001$). In cytokine storm, platelet adhesion values remained low in coated COBRA-PzF ($1.78 \times 10^7 \pm 0.38 \times 10^7$) compared to all other devices (uncoated COBRA: $5.92 \times 10^7 \pm 0.96 \times 10^7$; Resolute Onyx: $7.27 \times 10^7 \pm 1.82 \times 10^7$; Synergy: $11.28 \times 10^7 \pm 1.08 \times 10^7$; $p \leq 0.0001$). Although cytokine storm conditions significantly increased neutrophil activation in all stents, it was significantly less in coated and uncoated COBRA, and in Resolute Onyx than in Synergy.

Conclusions: Blood-biomaterials interactions may determine the thrombogenic potential of stents. Under simulated cytokine storm conditions, fluoropolymer-coated stents showed the most favorable anti-thrombogenic and anti-inflammatory properties.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has resulted in considerable morbidity and mortality throughout the world. 20%–30% of patients with this coronavirus disease 2019 (COVID-19) have evidence of cardiac injury defined as decline in ejection fraction or troponin I elevation, which is associated with an even higher mortality [1]. Severe SARS-CoV-2 infection is associated with overproduction of cytokines ("cytokine storm"). Notably interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) surge during the illness and decline during recovery [2]. Both IL-6 and TNF-α have been implicated in promoting overexpression of tissue factor in platelets and macrophages [3,4], establishing a procoagulant shift in the hemostatic balance and promoting fibrin generation in severe inflammatory states.

Cytokine storm associated with SARS-CoV-2 causes hypercoagulation with excess risk of thrombotic events [5]. Patients presenting with ST-segment elevation myocardial infarction (STEMI) and concurrent COVID-19 infection had higher rates of multi-vessel thrombosis and stent thrombosis (ST) versus non-infected patients [6,7]. Despite its

Table 1
Stent characteristics.

	Coated COBRA-PzF	Uncoated COBRA	Resolute Onyx	Synergy
Manufacturer	CeloNova	CeloNova	Medtronic	Boston Scientific
Stent material	L-605 cobalt-chromium CoCr alloy	L-605 cobalt-chromium CoCr alloy	Platinum-iridium alloy core and cobalt-chromium alloy shell	Platinum-chromium PtCr alloy
Polymer coating	Polyzene-F [poly-bis (trifluoroethoxy) phosphazene]	None	BioLinx® (C10, C19, and polyvinyl-pyrrolidinone polymer blend)	Synchrony™ PLGA [poly (DL-lactide-*co*-glycolide)]; abluminally only
Strut thickness	71 μm	71 μm	81 μm	79 μm
Polymer thickness	0.050 μm	–	5.6 μm	4 μm
Drug component	–	–	Zotarolimus	Everolimus

clinical relevance, the performance of different stent biomaterials has never been explored in the setting of COVID-19 infection.

Here, we present cases from our stent database with and without COVID-19 infection who underwent stent implantation for STEMI and compare signs of inflammation and thrombosis in these stents. To evaluate which biomaterial has the least thrombogenicity and might be more beneficial for stent implantation during COVID-19, we simulated cytokine storm conditions in vitro and examined compared platelet and neutrophil adhesion to stents of different biomaterials.

2. Material and methods

2.1. Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

2.2. Selection and histological processing of human stented coronary arteries

We searched the CVPath human coronary stent database for patients with and without confirmed COVID-19 infection who underwent stent implantation within 48 h before death. Only stents of equal type were included.

For histological staining, stented arteries were processed as described previously [8]. Briefly, the stented artery segments were fixed in formalin, dehydrated in a graded series of ethanol, and embedded in methylmethacrylate polymer. Segments of 2- to 3 mm thickness

were sawed from each stent, and cross-sections of 4- to 6 μm thickness were cut from each of the segments on a Leica RM2155 rotary microtome equipped with a tungsten carbide blade, mounted on slides, and stained with H&E and Movat pentachrome.

2.3. In vitro experiments

We tested the relative thromboresistance of stents coated with a fluorinated polymer (i.e. polyzene-F in COBRA-PzF (CeloNova, Carlsbad, CA)) versus the BioLinx polymer (i.e. C10, C19 and polyvinyl-pyrrolidinone polymers in Resolute Onyx (Medtronic, Minneapolis, MN)), and versus a bioabsorbable polymer (i.e. polylactic-*co*-glycolic acid in Synergy (Boston Scientific, Marlborough, MA)) (Table 1). Human blood was treated with TNF-α and IL-6 at levels consistent with what is seen in severe COVID-19 infections. Untreated blood served as control. The blood was circulated in a flow loop model, and stents were examined using confocal microscopy.

2.4. Silicone tube flow loop and stent deployment

Flow loops were assembled from silicone tubing (Cole-Palmer), connected via hose barb connectors (Cole-Parmer) (Fig. 1). Coronary stents were deployed within the tubing based on the manufacturer's suggested nominal pressure to obtain a stent-to-tube ratio of 1.1:1.0. Coated and uncoated COBRA stents were compared with Resolute Onyx stents (*n* = 4 experimental runs) and with Synergy stents (n = 4 experimental runs). Each flow loop contained only one stent at a time, so one experimental run comparing 3 different types of stents
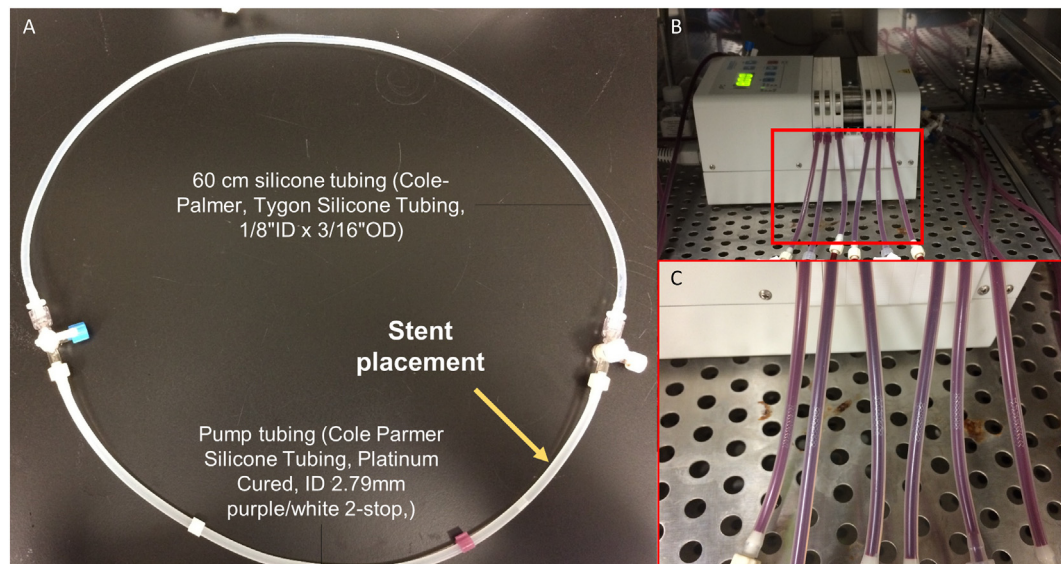
Fig. 1. Experimental setup. A) Each flow loop was assembled from silicone tubing. B) Whole blood was circulated for 60 min using a perfusion pump. C) Enlarged view on the stents deployed in silicone tubes.

(coated COBRA-PzF vs. uncoated COBRA vs. Resolute Onyx and coated COBRA-PzF vs. uncoated COBRA vs. Synergy) comprised 6 flow loops in a side-by-side experimental design (control vs. cytokine storm conditions, for each stent).

2.5. Preparation of human whole blood

Ethical approval was obtained by the Institutional Review Board at CVPath Institute. Whole blood (60 mL) was collected by venipuncture from healthy volunteers, none of whom was on anti-platelet therapy or other regular prescription. Blood was mixed with sodium citrate to obtain a final concentration of 0.32%. To simulate cytokine storm conditions, blood was supplemented with 400 pg/mL IL-6 (PeproTech) and 100 pg/mL TNF-α (Sigma Aldrich). Cytokine concentrations were chosen in accordance with the highest blood levels of severe COVID-19 cases that have been published [9–11], rounded up to the nearest 100.

Each flow loop was filled with whole blood and connected to a perfusion pump (ISMATEC). Blood was circulated at a flow rate of 35 mL/min for 60 min at 37 °C, 5% CO_2 (Fig. 1).

2.6. Sample fixation

Stented tubing pieces were washed in PBS and immersion-fixed in Zamboni's fixative (American MasterTech Scientific, Inc.) for 20 min. Stents were bisected longitudinally and placed in 15% sucrose in PBS at 4 °C overnight.

2.7. Immunofluorescence staining

One half of each stent was immunostained for adherent platelets using antibodies against CD42b (abcam) and CD61 (Immunotech). Staining against myeloperoxidase (MPO), which is released by activated
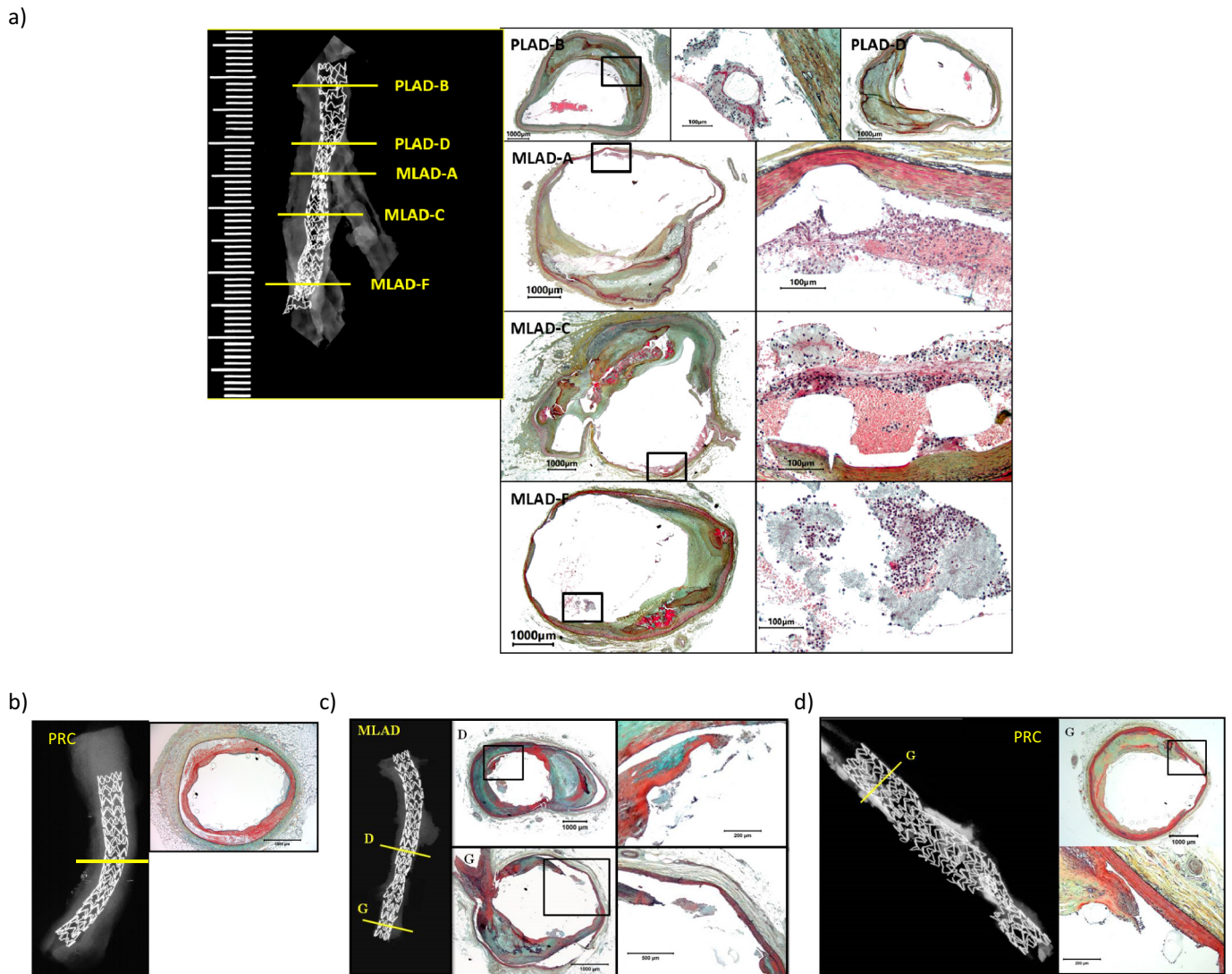

Fig. 2. Histopathology of drug-eluting stents, implanted for acute myocardial infarction within 48 h before death. A) 3.0 × 34 mm CoCr-EES stent implanted in the left anterior descending artery of an 81-year-old man who presented with COVID-19 positivity and STEMI. Subject was provided with mechanical ventilator support but developed cardiogenic shock and expired 1 day after admission. Yellow lines indicate approximate levels of histologic sections; boxed areas show fibrin-platelet thrombi around stent struts. Note the abundance of inflammatory cells around the stent struts, which is unusually high compared with stents of similar type and implantation duration in patients without COVID-19 (B–D). B) 3.0 × 23 mm CoCr-EES, implanted in the proximal right coronary artery of a 32-year-old female who was diagnosed with acute inferior wall myocardial infarction due to dissection of the proximal right coronary artery. The patient became unresponsive during procedure and could not be resuscitated. Note the absence of inflammation around struts most of which are well apposed. C) 3.0 × 28 mm CoCr-EES stent, implanted in mid left anterior descending artery of a 76-year-old woman who presented with STEMI. The patient's condition deteriorated over the next two days with acute kidney injury and pulseless electric activity arrest. Sections show uncovered struts and focal areas of plaque disruption, but thrombi and inflammatory cells are practically absent. D) 3.0 × 27 mm CoCr-EES stent placed in the proximal right coronary artery of a 58-year-old male who died shortly after stenting procedure. The section shows plaque disruption and limited medial dissection but absence of inflammation. PLAD, proximal left anterior descending; MLAD, mid left anterior descending; PRC, proximal right coronary.

neutrophils [12], was performed in the other halves of each stent (Dako). The antibodies were visualized by secondary antibodies conjugated to an Alexa Fluor® 555 fluorophore (both Invitrogen). The nuclear counterstain was DAPI (Invitrogen).

2.8. Image quantification

After immunostaining, stents were mounted 'en face' on glass slides and coverslipped using aqueous mounting media. For platelet immunofluorescence, the entire surface of the stent was scanned (10× objective) (Zeiss, LSM 880, Zen Black software, 2.3 SP1). Representative

high-power images were taken of 3 randomly selected struts from the proximal, mid, and distal part of each stent half using a 20× objective. Exported representative 20× JPG images were imported into Nikon NIS-Elements Advanced Research analysis software. To correct for possible photobleaching and other confocal error sources, image parameters were normalized by correcting the maximum pixel intensity value present to 255, and the average background value to 0. A stock noise reduction filter was applied uniformly across all images. A general analysis tool was created in NIS-Elements which applies a binary layer to the red channel, to pixels in the intensity range 40 to 255. The general analysis calculates the total pixel area with a red channel signal above
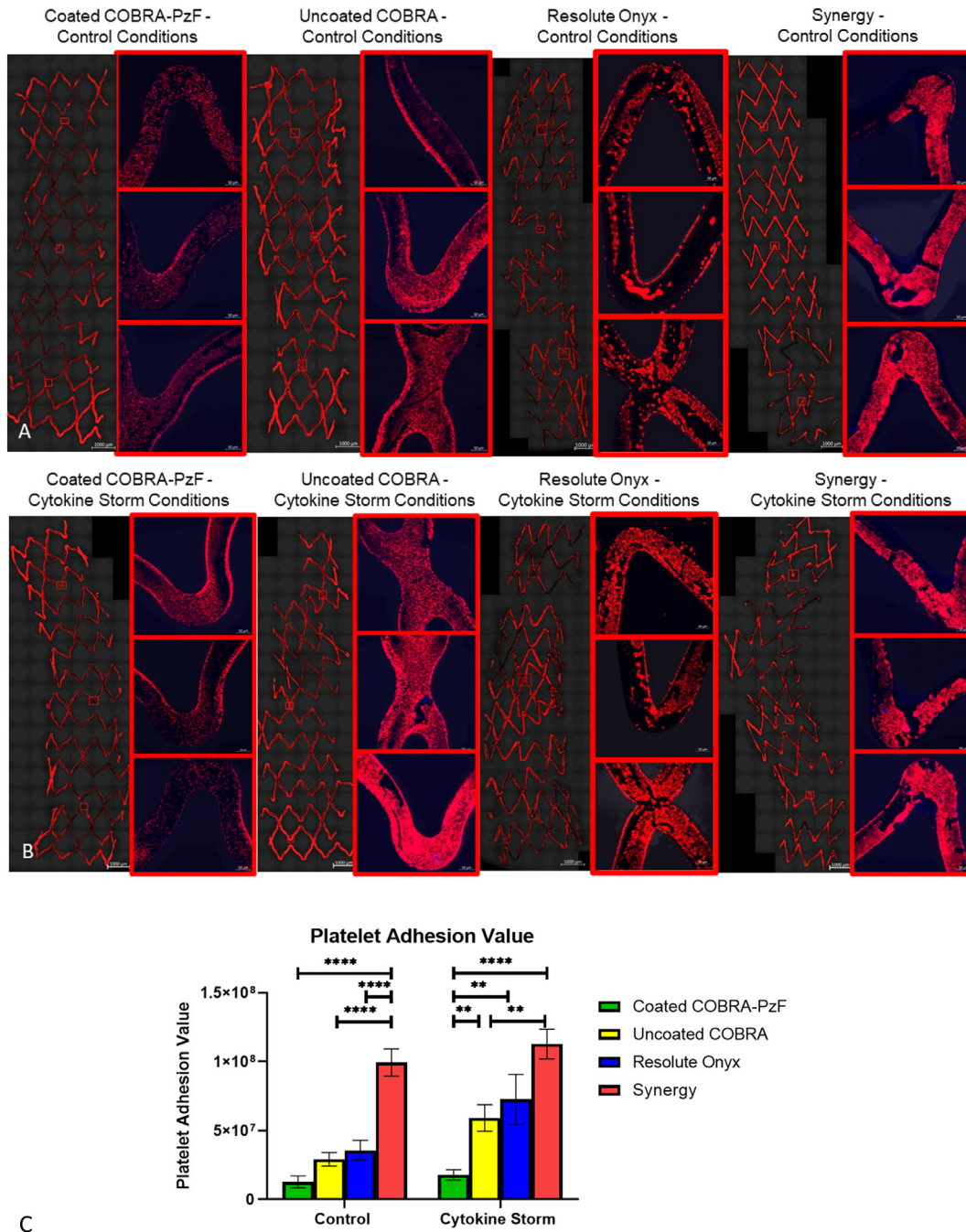
Fig. 3. Platelet adhesion. Representative images of platelet adhesion, assessed by immunodetection of surface antigens CD61 and CD42b (red channel) under control (A) and cytokine storm conditions (B), derived from confocal microscopy (10× and 20× magnifications). A + C) Under control conditions, platelet adhesion was significantly lower in coated and uncoated COBRA and Resolute Onyx than in Synergy. B + C) In cytokine storm, platelet adhesion remained significantly lower in coated COBRA-PzF compared to all other devices evaluated.

40 and calculates the sum of the intensities-per-pixel of every pixel covered by that binary. Total strut area was calculated, and the area fraction covered by platelets or neutrophils was calculated by dividing coverage area by total strut area. This area fraction was multiplied by the summed
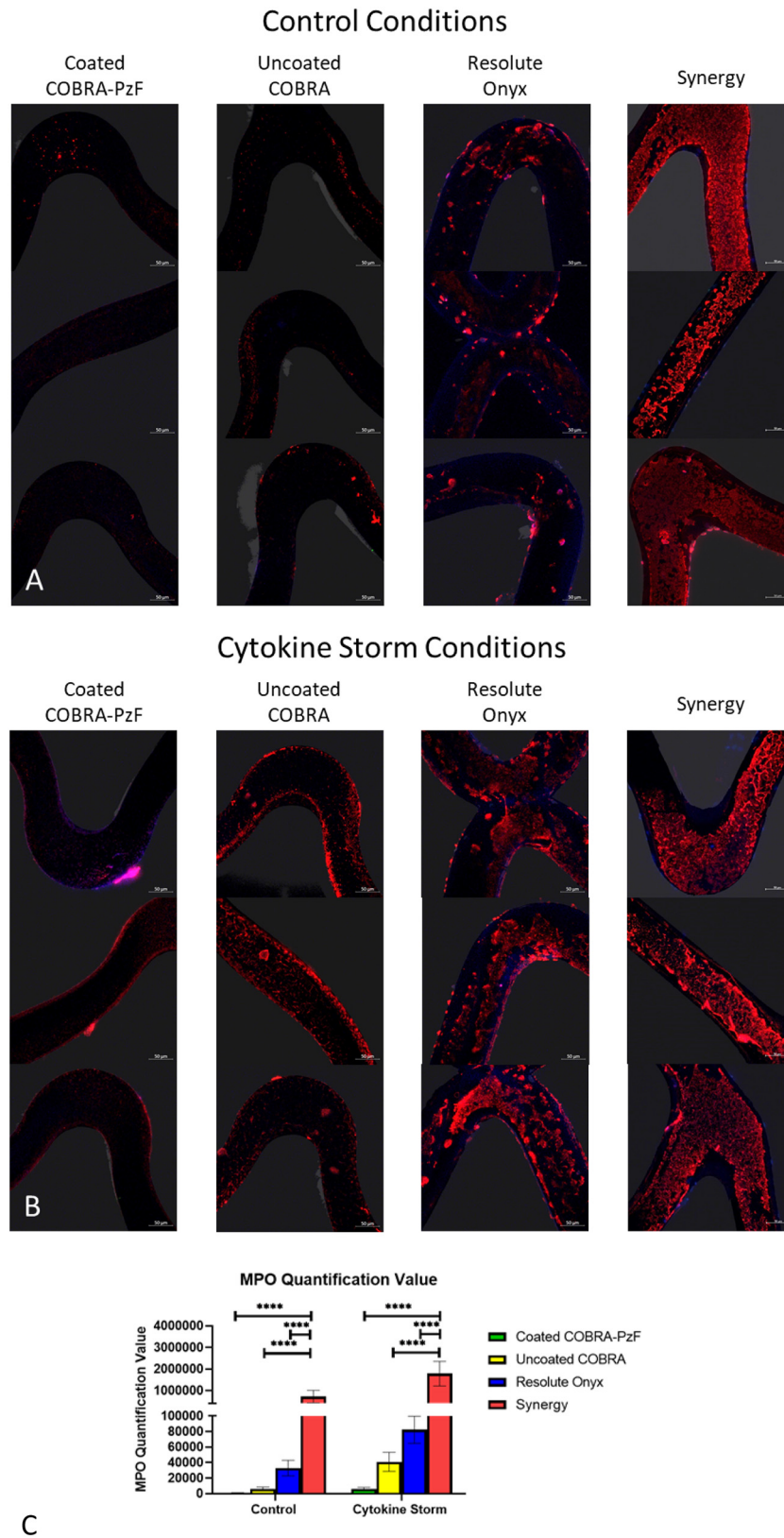
Fig. 4. Comparison of neutrophil activation in coated and uncoated COBRA, Resolute Onyx, and Synergy. A + B) Representative images derived from confocal microscopy (20× magnification). MPO deposition was very low in coated and uncoated COBRA, and lower in Resolute Onyx than in Synergy, both under control (A) and under cytokine storm conditions (B). C) MPO quantification values were significantly higher in Synergy versus coated and uncoated COBRA, and versus Resolute Onyx, both under control conditions and under cytokine storm conditions. There was no difference in neutrophil activation between the coated and uncoated version of COBRA.

Coated COBRA-PzF

Control Conditions | Cytokine Storm Conditions
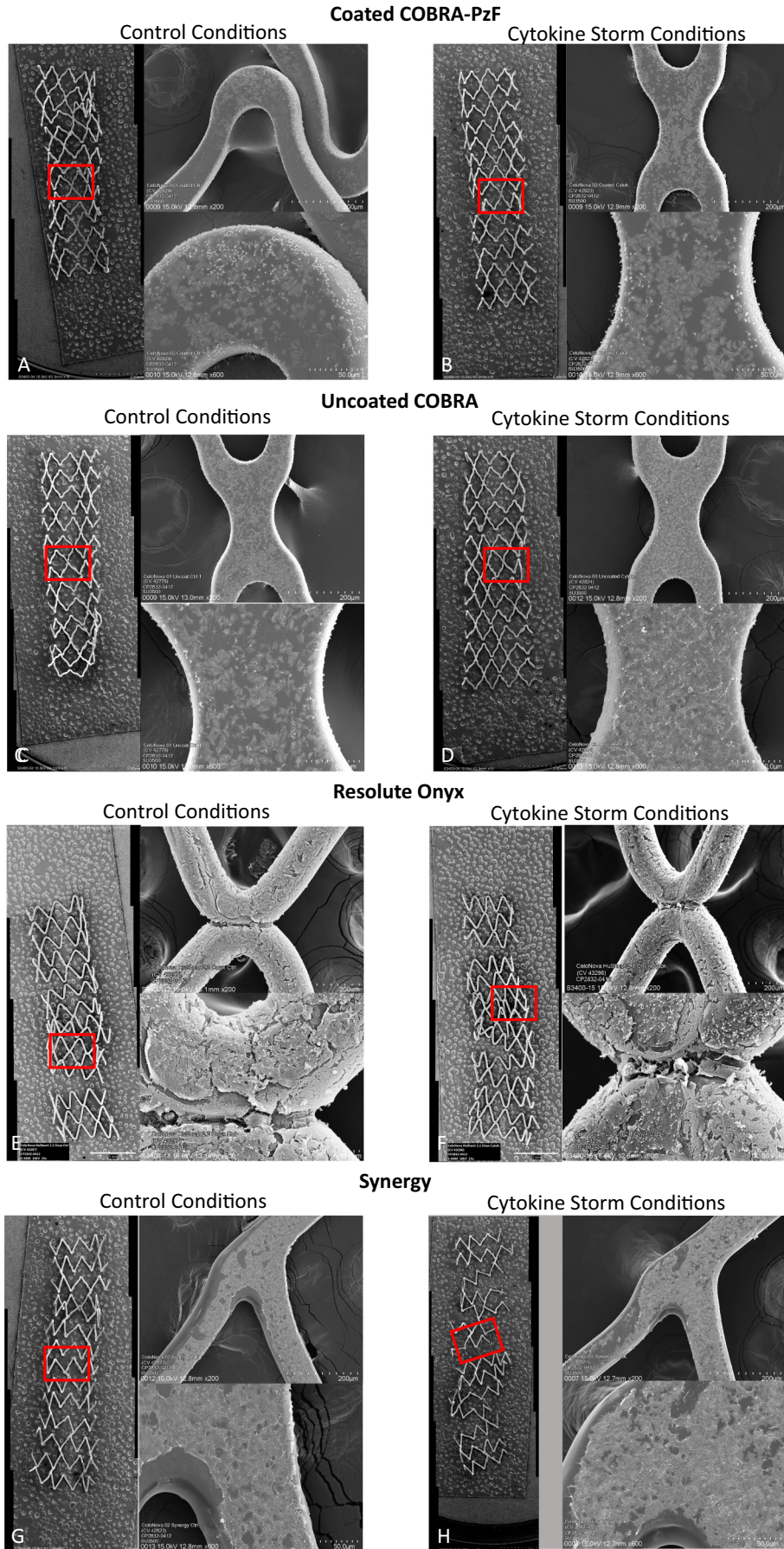
intensity to generate an intensity per coverage area metric (Coverage Area Fraction*Sum Red Channel Intensity), referred to as "Platelet Adhesion Value" and "MPO Quantification Value". Representative images with obvious and un-correctable imaging defects such as visible flaking, photobleaching, or out-of-plane Z axis artifacts were excluded from quantitation.

2.9. Statistical analysis

Data are expressed as means $\pm$ SEM. The data were statistically analyzed using GraphPad Prism software (version 8.4.3). Shapiro-Wilk test was used to check normality. Overall group means were compared using one-way ANOVA, followed by Tukey's multiple comparisons test, and differences between cytokine storm and control conditions in each stent were compared using Student's t-test. A value of $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Human autopsy cases of DES in COVID-19 and non-COVID-19 patients who died shortly after implantation

We consulted our database for autopsy cases with and without COVID-19 who underwent stent implantation for STEMI within 48 h before death and had patent stents at the time of death. Only stents of equal type and similar implantation duration were included. Our search yielded one 3.0×34 mm cobalt-chromium (CoCr) everolimus-eluting stent (EES) implanted for STEMI in a patient with severe COVID-19 infection and three CoCr-EES (3.0×23 mm, 3.0×28 mm, and 3.0×27 mm) that were implanted in patients without COVID-19. While we observed unusually high adhesion of inflammatory cells and platelets to the stent struts, but no ST, in the patient with confirmed COVID-19 infection (Fig. 2a), none of the patients without COVID-19 showed signs of platelet accumulation or severe inflammation around the stent struts (Fig. 2b–c). Although limited in number, these observations suggest COVID-19 infection may induce a pro-inflammatory and pro-thrombogenic environment. Prior reports suggested an increased risk of ST in COVID-19 patients presenting with STEMI [13–19], suggesting COVID-19 may affect vascular responses to stenting.

In order to explore this hypothesis, we evaluated the thrombogenic and inflammatory potential of different types of stents during a simulated cytokine storm induced by COVID-19 using a novel in vitro flow loop. To simulate cytokine storm conditions, blood was supplemented with 400 pg/mL IL-6 and 100 pg/mL TNF-α. These concentrations were chosen in accordance with the highest blood levels seen in severe COVID-19 cases that have been published [9–11]. Each flow loop was filled with whole blood and connected to a perfusion pump (ISMATEC). Blood was circulated at a flow rate of 35 mL/min for 60 min at 37 °C, 5% CO_2 (Fig. 1). We compared the relative thromboresistance of fluorinated polymer-coated stents (COBRA-PzF) versus BioLinx polymer-coated stents (Resolute Onyx) and versus an abluminally bioabsorbable polymer-coated stent (Synergy) (Table 1).

3.2. Platelet adhesion assessment

First, platelet adhesion was analyzed under control vs. cytokine storm conditions in each stent (Supplemental Table 1). While cytokine storm conditions did not enhance platelet adhesion in coated COBRA-PzF, we observed significantly greater platelet adhesion under cytokine storm conditions compared with control in uncoated COBRA

stents, and a trend towards higher platelet adhesion under cytokine storm conditions in Resolute Onyx. Platelet adhesion in Synergy did not differ from control under cytokine storm conditions and was higher than for all other stent tested regardless of condition.

Next, we compared all four devices under control conditions and under cytokine storm conditions, respectively (Fig. 3). Under control conditions, platelet adhesion was significantly lower in coated COBRA-PzF, uncoated COBRA, and Resolute Onyx than in Synergy. No differences were seen between Resolute Onyx and coated and uncoated COBRA. Under cytokine storm conditions, platelet adhesion remained low in coated COBRA-PzF, with significantly lower platelet adhesion values than in all other stents tested. Platelet adhesion in cytokine storm was similar in uncoated COBRA and Resolute Onyx, and – as opposed to control conditions - there was no difference between Resolute Onyx and Synergy under cytokine storm conditions anymore.

3.3. Neutrophil activation assessment

We also evaluated neutrophil activation under control and cytokine storm conditions by staining against MPO (Supplemental Table 2). Cytokine storm enhanced neutrophil activation in coated and uncoated COBRA stents, and in Resolute Onyx, albeit to a modest level (compared to its effect on Synergy). In Synergy, we observed tendencies towards enhanced neutrophil activation under cytokine storm conditions compared with control, but without statistical significance.

When directly comparing the devices, neutrophil activation was significantly higher in Synergy versus coated and uncoated COBRA stents, and versus Resolute Onyx, both under control and under cytokine storm conditions (Fig. 4). There was no difference between the coated and uncoated version of COBRA. Enhanced cell adhesion to Synergy as compared to coated and uncoated COBRA and Resolute Onyx stents was confirmed by SEM imaging (Fig. 5).

4. Discussion

The purpose of this study was to assess acute thrombogenicity and inflammatory potential of different stent biomaterials under control and COVID-19-simulated cytokine storm conditions in an in vitro flow loop using human whole blood.

4.1. Effect of cytokine storm on coagulation and thrombogenicity

Elevated cytokine levels have been found in patients with COVID-19-associated pneumonia [20]. Cytokine storm was simulated by the addition of IL-6 and TNF-α to human whole blood. To simulate a worst-case scenario, the dosages of 400 pg/mL IL-6 and 100 pg/mL TNF-α were chosen in accordance with the highest blood levels of these cytokines in patients with COVID-19 that have been published [9–11]. A study reporting data on 389 confirmed COVID-19 patients from Wuhan, China, reported IL-6 levels up to 160 pg/mL and TNFα levels up to 31 pg/mL even in patients with mild disease, while IL-6 rose to 300 pg/mL and TNFα went up to 41 pg/mL in patients with severe disease [10]. Likewise, IL-6 levels ranged between 68 and 333 pg/mL in a single-center study reporting clinical features of the first 50 COVID-19 patients admitted to a German tertiary hospital [11]. Another study reported serum IL-6 levels up to nearly 2000 pg/mL in 54 patients with COVID-19 infection and macrophage activation syndrome or immune dysregulation, while TNFα levels ranged between 8 and 37 pg/mL even in patients with only intermediate functional state of the immune system [9].

Fig. 5. Representative scanning electron microscopy images (15×, 200×, and 600× magnifications). A) Only a moderate number of cells is attached to coated COBRA-PzF under control conditions, and cell adhesion was not enhanced in cytokine storm (B). C) While a comparatively low number of cells adhered to uncoated COBRA under control conditions, cytokine storm significantly enhanced cell adhesion (D). E) Resolute Onyx stents were covered with a layer of plasma proteins, inflammatory cells, and platelets under control conditions, and cell adhesion was not further enhanced under cytokine storm conditions (F). G) Cell adhesion to Synergy was already high under control conditions and was not further enhanced by cytokine storm (H).

SARS-CoV-2 infections are associated with coagulopathy ("COVID 19-associated coagulopathy", CAC), presenting with elevation of D-dimer and fibrin/fibrinogen-degradation products [21]. While the virus itself does not appear to intrinsically trigger coagulation, CAC is thought to derive from the profound inflammatory response [5] with excess production of pro-inflammatory cytokines contributing to the activation of coagulation [22]. Especially IL-6 and TNF-α are important mediators as they upregulate tissue factor on the cell surface [3,4], which initiates the extrinsic pathway of coagulation [23]. Furthermore, TNF-α suppresses endogenous anticoagulant pathways [4]. Hence, IL-6 and TNF-α establish a procoagulant shift in the hemostatic balance.

4.2. Relationship between COVID-19 and myocardial infarction

Patients with coronary artery disease (CAD) and those with cardiovascular risk factors are at increased risk for MI during infections [24]. Elevated troponin I levels have been found in 8%–28% of patients with COVID-19 [25,26]. With surging numbers of COVID-19 patients, and given the high prevalence of CAD, reasonable numbers of patients with combined STEMI and COVID-19 can be expected. Primary PCI remains the gold standard of care for STEMI patients during the COVID-19 pandemic [27]. However, the procoagulant state of COVID-19 patients may trigger acute ST. While the event of ST in STEMI patients who have tested positive for COVID-19 has been described in a number of case reports [13–19], only two larger multicenter studies have been published so far. The first multicenter study, published in July 2020, included 78 patients with STEMI and COVID-19. Stent thrombosis occurred in 4 out of 19 patients treated with primary PCI (21%) [28]. Another study compared in-hospital outcomes of 1010 consecutive STEMI patients with and without COVID-19 and reported a higher incidence of stent thrombosis in COVID-19 patients (3.3% vs. 0.8%; $p = 0.020$) [6].

Indeed, even though the case presented did not have a ST, we observed unusually high adhesion of inflammatory cells and platelets to the struts in a stent implanted for STEMI in a patient with severe COVID-19 infection, while inflammation was practically absent around struts of the same type of stent in patients without COVID-19. We acknowledge the fact that findings in a single patient can only be hypothesis generating, however, in conjunction with the increased risk of ST in clinical studies, we assume the use of a stent that is less thrombotic and less likely causing inflammatory reactions might be beneficial in a situation of STEMI and simultaneous COVID-19 infection, but this needs to be confirmed in clinical trials.

4.3. Polymer coatings affect stent thrombogenicity, inflammation, and endothelialization

Cytokine storm triggered platelet adhesion in uncoated COBRA stents, and in Biolinx-coated stents, while it was already high under control conditions in stents abluminally coated with bioabsorbable polymer. Furthermore, we observed enhanced neutrophil activation in all stents under cytokine storm conditions. These experiments confirmed our hypothesis that COVID-19-induced cytokine storm seems to affect the interaction of stents with blood. We saw more inflammation and platelet accumulation, consistent with our clinical case.

Fluoropolymers are known to have anti-thrombotic properties. Based on a CoCr alloy, COBRA-PzF has a nano-thin coating of poly-bis (trifluoroethoxy)phosphazene (Polyzene-F or PzF) which has been shown to preferentially adsorb albumin instead of coagulation-stimulating proteins [29]. Less platelet adhesion was observed in COBRA-PzF as compared to conventional drug-eluting stents (DES) in a pig shunt study [30], and clinical studies reported low incidences of ST and spontaneous MI [31–34]. On the other hand, hydrophilic polymer surfaces, such as BioLinx in Resolute Onyx, have been suggested to have superior biocompatibility compared with hydrophobic polymer coatings, mostly because they do not induce monocyte adhesion [35].

Consisting of an outer shell of CoCr and a platinum-iridium inner core, Resolute Onyx showed particularly low rates of ST [36]. Bioabsorbable polymers are often cited as an alternative to biostable polymers for DES coatings. Synergy is an EES on a platinum-chromium (PtCr)-based platform with abluminal bioabsorbable polymer coating which demonstrated accelerated vascular healing and minimal inflammation in animal studies [37] and excellent outcomes in randomized trials [38].

When comparing these devices in our study, coated COBRA-PzF showed the most favorable anti-thrombogenic and anti-inflammatory properties, both under control and under cytokine storm conditions, whereas platelet adherence and neutrophil activation were highest in Synergy. While cytokine storm conditions enhanced platelet adhesion in uncoated COBRA and in Resolute Onyx, we did not observe significant differences between control and cytokine storm conditions in coated COBRA-PzF and in Synergy. Platelet adherence in Synergy, however, was already high under control conditions, and cytokine storm might not have had the ability to further increase it. In contrast, platelet adhesion was overall low in coated COBRA-PzF, suggesting that the anti-thrombogenic properties can be attributed to its PzF-nanocoating.

The question why even the uncoated COBRA stent had less platelet and neutrophil adhesion than the bare luminal surface of Synergy may be related to differential stent platforms. COBRA stents have thinner struts than Synergy (71 μm vs. 79 μm plus the 4 μm-abluminal polymer coating), which is an important determinant of thrombogenicity [39]. Furthermore, the metal alloy is different in COBRA and Synergy. While COBRA is manufactured from a CoCr alloy, PtCr is used in Synergy. Recent data from 7045 patients suggested that Synergy was associated with a higher risk of acute ST when compared with a CoCr-based EES (1.2% vs. 0.3%, $p = 0.032$) [40]. Of note, 14 of 15 acute ST events among those treated with Synergy, occurred in high-risk patients.

5. Limitations

Our study has several limitations. First, this is a pure benchwork study, and we did not confirm our findings in animals. Second, although cytokine storm is considered the major determinant for thrombogenicity in SARS-CoV-2 infections, we did not perform the experiments with blood from COVID-19-infected patients. Thus, we might have missed the potential impact of other blood components potentially playing a role in CAC. In addition, although IL-6 and TNF-α are believed to be the most important players in COVID-19 induced cytokine storm [41,42], we excluded other cytokines which may have additional effects on stent thrombogenicity. Third, we compared platelet and neutrophil adhesion in a select set of stents only, all of which, however, were specifically designed to reduce inflammation and to enhance vascular healing after PCI [35,43,44]. Fourth, stents were implanted in non-endothelialized silicone tubes, missing the important pro- or anticoagulant impact of endothelial cells. Considering these important limitations, the findings of our study can only be hypothesis generating, and not practice changing.

Furthermore, the histopathology comparison between stents in COVID-19-infected and non-COVID-19-infected patients did not allow for representative statistical evaluation because of the small sample size given the limited availability of stent samples obtained from autopsy of COVID-19 cases. Nevertheless, to the best of our knowledge, histopathology data from patients with acute stent implantation during COVID-19 infection have never been published before, and our findings emphasize the clinical relevance of evaluating thromboresistance of stents in a situation of cytokine storm.

6. Conclusions

Nanocoated COBRA-PzF showed the most favorable anti-thrombogenic and anti-inflammatory properties, both under control and under cytokine storm conditions, whereas platelet adherence and neutrophil activation were highest in Synergy. We assume the anti-thrombogenic properties can be attributed to the unique Polyzene-F

nanocoating, preventing platelet adhesion even in an in vitro simulated cytokine storm.

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CRediT authorship contribution statement

Anne Cornelissen: Conceptualization, Methodology, Investigation, Writing – original draft. **Matthew Kutyna:** Formal analysis, Investigation. **Qi Cheng:** Visualization. **Yu Sato:** Methodology, Writing – review & editing. **Rika Kawakami:** Writing – review & editing. **Atsushi Sakamoto:** Writing – review & editing. **Kenji Kawai:** Writing – review & editing. **Masayuki Mori:** Writing – review & editing. **Raquel Fernandez:** Writing – review & editing. **Liang Guo:** Writing – review & editing. **Dario Pellegrini:** Resources, Investigation. **Giulio Guagliumi:** Resources, Investigation. **Mark Barakat:** Conceptualization, Resources, Investigation, Writing – review & editing. **Renu Virmani:** Writing – review & editing. **Aloke Finn:** Supervision, Writing – original draft.

Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carrev.2021.03.023.

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