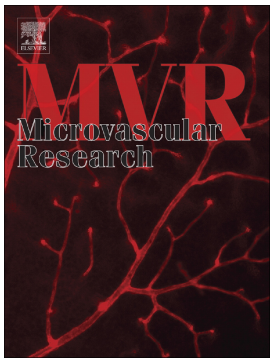

Microvascular changes following exposure to iodinated contrast media in vitro. A qualitative comparison to serum creatinine concentrations in post-cardiac catheterization patients

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Microvascular changes following exposure to iodinated contrast media in vitro. A qualitative comparison to serum creatinine concentrations in post-cardiac catheterization patients.

Magnusson Marine[1], Gerk Ulrich[2], Schüpbach Gertraud[3], Rieger Juliane[4], Plendl Johanna[5], Marin Ilka[5], Drews Barbara[1], Kaessmeyer Sabine[1]

[1] Division of Veterinary Anatomy, Vetsuisse Faculty, University of Bern, Bern, Switzerland.
[2] Städtisches Klinikum Dresden, Dresden, Germany
[3] Veterinary Public Health Institute, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[4] Institute of Translational Medicine for Health Care Systems, Department of Human Medicine, Faculty of Medicine, MSB Medical School Berlin, Berlin, Germany
[5] Institute of Veterinary Anatomy, Department of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany

Corresponding author: Kaessmeyer Sabine, Division for Veterinary Anatomy, Vetsuisse Faculty, University of Bern, Länggassstrasse 120, Postfach, CH-3001 Bern,
sabine.kaessmeyer@unibe.ch

Highlights

- Similarities between results of *in vitro* and *in vivo* methods were observed.
- *In vitro*, changes occurred 3 days after radiological contrast media (RCM) exposure.
- *In vivo*, serum creatinine concentration increased 3 days after RCM exposure.
- *In vitro*, EDTA, but not Trometamol, induced changes similar to RCM.

Abstract

Introduction. Contrast-associated acute kidney injury (CA-AKI) is characterized as a loss of renal function following radiological contrast media administration. While all contrast media induce variable changes in microvascular endothelial cells *in vitro*, only few studies report clinical significance of their findings. A comprehensive assessment of the effect of iodinated contrast media on the renal function *in vitro* and *in vivo* is essential. The aim of our study was to morphometrically quantify the effect of two different contrast media (Iobitridol and Iodixanol) on vascular endothelial capillaries *in vitro* and to analyze their effect on the renal function of patients who underwent cardiac catheterization including the intra-arterial administration of contrast media, by measuring serum creatinine concentration (SCr), a byproduct of muscle metabolism, primarily excreted by the kidneys. Our hypothesis suggests that conducting a qualitative comparison of both outcomes will enable identification of differences and similarities between *in vitro* and *in vivo* exposure.

Material and Methods. *In vitro*, co-cultures of human dermal fibroblasts and human dermal microvascular endothelial cells forming capillary beds were exposed to a mixture of phosphate buffered saline and either Iobitridol, Iodixanol, or one of their supplements EDTA or Trometamol for 1.5 or 5 minutes. Negative control co-cultures were exposed exclusively to phosphate buffered saline. Co-cultures were either directly fixed or underwent a regeneration time of 1, 3 or 7 days. An artificial intelligence software was trained for detection of labelled endothelial capillaries (CD31) on light microscope images and measurements of morphometric parameters. *In vivo*, we retrospectively analyzed data from patients who underwent intra-arterial administration of contrast media and for whom SCr values were available pre- and post-contrast exposition (1, 3, and 7 days following procedure). Temporal development of SCr and incidence of CA-AKI were assessed. Both exposure types were qualitatively compared.

Results. *In vitro*, Iobitridol, Iodixanol and EDTA induced a strong decrease of two morphometric parameters after 3 days of regeneration. *In vivo*, a significant increase of SCr and incidence of CA-AKI was observed 3 days following procedure in the post-contrast media patients. No difference was observed between groups.

Discussion. Two of the morphometric parameters were inversely proportional to the SCr of the patients. If the endothelial damages observed *in vitro* occur *in vivo*, it may result in renal hypoxia, inducing a loss of kidney function clinically translated into an increase of SCr. Further development of our *in vitro* model could allow closer replication of the internal structure of a kidney and bridge the gap between *in vitro* studies and their clinical findings.

Keywords: radiological contrast media, microvascular endothelial tubes, Iodixanol, Iobitridol, acute kidney injury, serum creatinine, cardiac catheterization, co-cultures

AI	Artificial intelligence
ANOVA	One-Way analysis of variance
CA-AKI	Contrast-associated acute kidney injury
CI	Confidence interval
DMEM	Dulbecco's modified Eagle medium
EDTA	Ethylenediaminetetraacetic acid
Fb	Fibroblasts
HKMEC	Human kidney microvascular endothelial cells
HMVEC-D	Human dermal microvascular endothelial cells
IOCM	Iso-osmolar contrast media
LOCM	Low-osmolar contrast media
PBS	Phosphate buffered saline
RCM	Radiological contrast media
SCr	Serum creatinine concentration
SD	Standard deviation
Trometamol	Tris(hydroxymethyl)aminomethane

1 Introduction

Contrast-associated acute kidney injury (CA-AKI) is characterized as a decrease in renal function following intravascular administration of iodinated radiological contrast media (RCM) (Palevsky et al., 2013). The loss of renal function is clinically characterized as an increased concentration of serum creatinine (SCr), a byproduct of muscle metabolism, primarily excreted by the kidneys ("Section 2," 2012).

Although the pathogenesis of CA-AKI is multifactorial and still not fully understood. Three main factors contributing to CA-AKI are discussed: direct toxicity on epithelial tubular cells and endothelial cells, hemodynamic changes, and oxidative stress (Berg, 2000). These factors affect different structures in the kidney, including the tubules and the microvasculature. Regarding the microvasculature, the direct cytotoxicity of RCM on endothelial cells leads to pinocytosis (osmotic nephrosis), oxidative stress, and apoptosis. Furthermore, dysregulation of vasoactive substances such as nitric oxide or adenosine by RCM disturbs hemodynamic mechanisms and leads to prolonged vasoconstriction of the renal vessels. The resulting hypoperfusion causes hypoxia in the kidney, which leads to loss of renal function (Cho and Ko, 2022; Seeliger et al., 2012; Sendeski, 2011).

In an effort to better understand the pathophysiology of CA-AKI, the effects of iodinated RCM on endothelial cells have been assessed *in vitro*. The cellular damage and regeneration were measured by the quantification of morphological changes of endothelial cells (Kaessmeyer et al., 2017a) and/or cell-viability and apoptosis factors (Franke et al., 2013; Ramponi et al., 2007; Ren et al., 2017; Zhang et al., 2000). While all RCM induced changes in varying degrees *in vitro*, the translation of these results to clinical practice remains problematic and only few *in vitro* studies report clinical significance of their findings (Franke et al., 2011; Heyman, 2005; Zhang et al., 2018).

In vivo, the safety of different types of RCM has been tested, depending on their osmolality, viscosity and whether they are ionic or not. Due to their higher nephrotoxicity, ionic high-osmolar contrast media are no longer in use in human medicine and have been replaced by safer non-ionic low-osmolar (LOCM) and iso-osmolar contrast media (IOCM) (Hill et al., 1993; Rudnick et al., 1995). However, CA-AKI is still reported following injection of RCM and several *in vivo* studies have highlighted both types of RCM (LOCM, IOCM) to induce changes in renal function, with lower changes associated with IOCM administration than LOCM administration (McCullough et al., 2021, 2006; Murakami et al., 1998; Roriz et al., 1999). More recently, randomized controlled clinical studies have challenged the nephrotoxicity of RCM, demonstrating no increased risk of developing CA-AKI in the exposed groups compared to control groups (McDonald et al., 2017, 2014; Williams et al., 2020). However, it remains crucial to consider independent (Davenport et al., 2013; Yildiz et al., 2019) and dependent risk factors, such as the administration route. Indeed, the intra-arterial route often performed in cardiac diagnostic and therapeutic procedures might increase the risk of CA-AKI (Ghumman et al., 2017; Schönenberger et al., 2019; Wagner et al., 2022; Wu et al., 2022). One of the explanations proposed in the literature is the higher peak RCM concentration delivered in the renal arteries (Gutierrez and Newhouse, 2017). Furthermore, emboli formed during the injection might cause blockage of the renal vessels, leading to hypoxia and, eventually to a loss of renal function (Eggebrecht et al., 2000). Given the high prevalence of cardiovascular diseases, cardiac catheterization including intra-arterial injections of RCM are frequent (Timmis et al., 2022). However, due to the lack of patients undergoing cardiac catheterization without RCM exposure, studies are limited in differentiating RCM-dependent from RCM-independent CA-AKI. Therefore, it remains crucial to understand the effect of iodinated RCM on endothelial cells

and renal function after intra-arterial administration by bridging the gap between *in vitro* studies and their clinical outcomes to determine the primary cause of CA-AKI. This is necessary to determine whether the protocol related to RCM administration need to be adapted when making decisions for specific care of patients at risk, considering that these decisions might be taken at the expense of diagnostic and/or therapeutic quality. Prior research on animal models *in vivo* showed interesting results (Zhao et al., 2011). Their experimental set up allowed them to directly compare *in vivo* endothelial injury markers with *ex vivo* histological findings. Nevertheless, care must be taken in extrapolating their results to humans, as animal models are not always representative of humans. In theory, this experimental set up could be applied to human patients. However, collection of kidney biopsies *intra vitam* or *post-mortem* for histological analysis would pose ethical and organizational challenges. Therefore, there is a necessity to create alternative experimental models that follow ethical guidelines, steer clear of animal research, and closely resemble real-life conditions.
The aim of our study was to compare the effect of two commonly used RCM, one LOCM and one IOCM on an *in vitro* model reproducing lumenized endothelial capillary bed. We sought to replicate the exposure time and the dose comparable to that experienced by endothelial cells in the kidney following intra-arterial administration. For this purpose, endothelial capillary beds in co-cultures were morphometrically analyzed directly after exposure and after specific regeneration time points. For the assessment of the clinical significance of our findings, SCr of patients exposed to the same RCM as part of a cardiac catheterization was analyzed *in vivo*. For both experimental settings, identical assessment time points following exposure to RCM were used. This experimental design allowed a qualitative comparison of the effect of RCM on endothelial cells *in vitro* and on the renal function in hospitalized patients, taking a first step towards the development and evaluation of a more complex *in vitro* kidney model.
In this study, the term "contrast-associated acute kidney injury" is employed to encompass all forms of AKI, including those resulting from causes unrelated to RCM. This is in contrast to the term "contrast-induced acute kidney injury" (CI-AKI) which specifically refers to AKI attributed to RCM administration in the absence of other causes.

2 Material and Methods

2.1 Radiological contrast media

Two RCM commonly utilized in cardiac catheterization were employed in their standard commercial formulations both for the *in vitro* and the *in vivo* experiments. These were Iobitridol (Xenetix®, Guerbet, Sulzbach, Germany, 300 mg I/ml, osmolality 695 mosm/kg H_2O, viscosity at 37°C 6,0 mPas·s, 0.1 mg/ml ethylenediaminetetraacetic acid (EDTA), 0.36 mg/ml tris(hydroxymethyl)aminomethane (Trometamol)) and Iodixanol (Visipaque®, GE Healthcare, Chicago, USA, 320 mg I/ml, osmolality 290 mosm/kg H_2O, viscosity at 37°C 11,1-11,8 mPas·s, 0.1 mg/ml EDTA, 1.2 mg/ml Trometamol).

2.2 *In vitro* experiments

2.2.1 Cells and culture conditions

Human dermal microvascular endothelial cells (HMVEC-D) from neonate foreskin (Lonza, Walkersville Inc., Walkersville, USA) were incubated in HMVEC-D differentiation medium (Lonza, Walkersville Inc., Walkersville, USA) according to supplier's instructions.
Human juvenile foreskin fibroblasts (Fb) from 3 donors were isolated from residual tissue of circumcision surgery according to Küchler et al. (Küchler et al., 2011) (patient consent obtained, ethics vote from Charité, Berlin, Germany, EA1/081/13). The cells were kindly provided by Prof. Sarah Hedtrich, Johanna Quandt-Professor at the Berlin Institute of Health at Charité (BIH). Fb were first cultured in "Fb medium": Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin (10'000U/ml) (all from Sigma-Aldrich, Taufkirchen, Germany) in a humidified atmosphere (37°C, 5% CO2). The medium was replaced every 2 to 3 days.

2.2.2 Generation of Fb/HMVEC-D co-cultures

In order to co-culture the Fb with the HMVEC-D, the Fb mono-cell cultures were stepwise primed to "HMVEC-D differentiation medium". Priming of the Fb mono-cell cultures to the HMVEC-D cell medium

was achieved over a 48-hour period. Therefore, fresh Fb medium was sequentially mixed with 25%, 50%, 75% and 100% of HMVEC-D cell medium and subsequently used as co-culture medium.
The Fb (5'000 cells per well) proliferated within 9 days in 24-well culture plates. Afterward 20'000 HMVEC-D cells were seeded on top of the Fb. Co-cultures underwent an incubation for 10 days in HMVEC-D cell medium, which was replaced every 2 to 3 days. Using this approach, it has formerly been demonstrated, that endothelial cells formed lumenized tubular structures (hereafter referred to as "tubes") (Kaessmeyer et al., 2017b). The tubes expanded, branched, and networked with each other, mimicking endothelial capillary beds.

2.2.3 Incubation with iodinated radiological contrast media or one of their supplements

After the 10-day co-culture period, the co-cultures were statically exposed to either one of the 2 iodinated non-ionic RCM (Iodixanol (Visipaque 320®) or Iobitridol (Xenetix 300®)) or to one of 2 supplements usually present in the commercial formulations of RCM (1.2 mg/ml Trometamol buffer or 0.1 mg/ml, EDTA). For exposure purpose, RCM and supplements, respectively (hereafter referred to as "test agents") were mixed with phosphate buffered saline (PBS) to produce a 30% v/v solution. This ratio was considered to be a reasonable concentration to mimic an *in vivo* intra-arterial injection (Franke et al., 2008; Kutt et al., 1963).
The exposure was performed as follows: first, the cell culture medium was removed, and the co-cultures were washed with prewarmed PBS. Afterwards the prepared mixture of the respective test agent and PBS was poured onto the co-cultures. The test agents remained static on the co-cultures for 1.5 minutes or 5.0 minutes (Franke et al., 2013, 2008; Kaessmeyer et al., 2017a). After exposure, one part of the co-cultures was washed with PBS and directly fixed (hereafter referred to as "no regeneration"). Remaining co-cultures were further incubated in cell culture medium after removal of the respective test agent and PBS and a washing step with PBS for a "regeneration" period of 1 day, 3 days, and 7 days, respectively before fixation. The incubation process following exposure to test agent will hereafter be referred to as "regeneration time". The regeneration times of 1 and 3 days were chosen based on the definition of CA-AKI as an increase in SCr observed 48 to 72h following RCM administration (Mehran and Nikolsky, 2006). To assess the further development of the morphometrical changes beyond 72 hours, a regeneration time of 7 days was added. For each combination of Fb donor, exposure time, regeneration time, and test agent, 3 to 4 co-culture replicates were exposed, processed, and analyzed. In addition, for each combination of Fb donor, exposure time, and regeneration time, 3 to 4 negative control co-cultures were cultured under similar conditions and exposed exclusively to PBS instead of PBS plus test agent.

2.2.4 Immunodetection of endothelial tubes

Directly following exposure, or after a regeneration period, respectively, co-cultures were washed with PBS, fixed in methanol/acetone (1:1) at -20°C for 10 minutes and dried at room temperature. Then, the cells were fixed with 4% neutral buffered formalin for 15 minutes at 4°C before being washed again with PBS and permeabilized with a mixture of PBS and octylphenoxypolyethoxyethanol (0.2% Triton-x-100®, Sigma-Aldrich, Taufkirchen, Germany). Finally, co-cultures were immersed in Protein Block Serum-Free (DAKO Diagnostika, Hamburg, Germany) for 1 hour at 4°C. The blocker was removed, and the endothelial cells were immunolabelled with CD31 (1:50 Monoclonal Mouse Anti-CD31 Endothelial Cell in blocking buffer, PECAM-1, Platelet endothelial cell adhesion molecule 1, DAKO, Diagnostika, Hamburg, Germany) overnight at 4°C.
Then, co-cultures were incubated with a horseradish peroxidase-labeled secondary antibody (1:60 Donkey-anti-Mouse-IgG-HRP in PBS) for 30 minutes at room temperature. The labeling was visualised with diaminobenzidine (Sigma-Aldrich, Taufkirchen, Germany). Nuclei were stained with hemalaun (Fluka-Chemie, Buchs, Switzerland). Negativ controls were performed in which the primary antibody was replaced by buffer or non-immune serum.

2.2.5 Image processing and selection of the images

A complete scan of each well was captured with a light microscope (DMi8 Leica Microsystems, Leica Camera AG, Wetzlar, Germany) and a digital camera (Leica MC 170 HD, Leica Camera AG, Wetzlar, Germany). Visual fields were standardized by cropping the scan to a size of 6 x 6 mm^2 (13'999 x 13'999 pixels; 0.43 µm/pixel) from each co-culture at a consistent location and magnification (10x) using a microscopic image acquisition software (Leica Application Suite X, Leica Camera Ag, Wetzlar, Germany).

Images were evaluated for presence of artefacts, such as background staining, air bubbles, foreign material, or poor contrast. If an artifact was considered excessively problematic by the investigator and posed a risk interfering with the study results, the image was excluded from the study. A total of 344 images was included in the study. Care has been taken to ensure the availability of images for at least two replicates from each combination (test agent, exposure time, regeneration time, Fb donor).

2.2.6 Training of an artificial intelligence for automated morphometric measurements

An artificial intelligence (AI) was trained using the NIS.ai Software (Nikon, Shinjuku, Tokyo, Japan) to allow automated segmentation of the endothelial tubes and morphometric measurements. The AI was trained with a selection of 24 crops from the light microscope images used in the study. Attention was taken to select images with a wide variety of properties, including different contrasts and different background colors. Images with artifacts were also included in the training to prevent the AI from confusing the latter with endothelial tubes, especially linear foreign structures.
Each of the 24 crops were split into 46 tiles of 2’048 x 2’048 pixels each. Manual binary segmentation of the endothelial tubes and sprouts was performed on 11 tiles from each of the 24 crops, resulting in a total of 264 tiles. Artifacts were not segmented. Once segmented, the 264 segmented crops were used for supervised machine learning of the AI, with a total of 1000 iterations.
An algorithmic workflow, developed on the NIS.ai Software, included automated labeling by the pre-trained AI, skeletonization of the tubes, and detection of branches. The skeletonization of the tubes was defined as a 1-pixel wide centerline representing the segmentation in a simplified form. The detection of branches was defined as the intersection of two or more skeletonized tubes or ramification of a skeletonized tube. The minimum length of the tubes detected by the AI was set to 0.43 µm, corresponding to the size of one pixel. Following these procedures, three morphometric parameters listed in Table 1 were incorporated into the workflow for automated measurements.

All 344 crops could be analyzed in series through the workflow. Results were automatically exported in an Excel file (Microsoft, Redmond, Washington, USA).

2.2.7 Statistical analysis

The parameters analyzed were described using mean value and standard deviation (SD). As all parameters were normally distributed, a One-Way analysis of variance (ANOVA) was used to compare exposed groups at individual time points. Repeated measures ANOVA was used to analyze the development of the parameters over regeneration time in the different exposed groups. In this study, statistical analyses were performed using the NCSS software (NCSS, Kaysville, Utah, USA). To account for multiple comparisons, the p-values were corrected using the Bonferroni Correction method. Given the analysis involved comparisons across 4 different test agents (2 RCM and 2 supplements) and 1 negative control group, as well as 4 different regeneration times, the significance threshold was adjusted to $p < 0.0031$ to maintain an overall Type I error rate of 0.05.

2.3 *In vivo* experiments

2.3.1 Subjects and radiological contrast media

The patient data were drawn from a database of all cardiac catheterization including an intra-arterial RCM injection performed at the cardiology department of the Städtisches Klinikum Dresden in Germany from January 2010 to October 2022. Due to the nature of the data source which consisted initially of a quality control list, individual patient consent was not obtained. The data utilized were thoroughly anonymized and de-identified to ensure patient confidentiality. A total of 7’628 cardiac catheterization were performed. The patients were included in the study if they (a) had either received Iobitridol (Xenetix 300®) or Iodixanol (Visipaque 320®) and (b) had available measurements of SCr pre- and post-contrast exposition (day 1, day 3 and day 7 following cardiac catheterization). SCr values measured before contrast exposition were established as the baseline SCr. Patients were excluded if they had undergone two or more cardiac catheterization within 7 days. In addition to regular measures, no specific measures were implemented for prophylaxis of CA-AKI. Choice of RCM was based on the availability in the clinic at the time of examination. RCM volume was weight based and protocol dependent.
Data were available for the genders "men" and "women". Data from other gender identities were not available. Hence, the terms "men" and "women" are used to refer to sex categories based on

biological characteristics, specifically male and female anatomy, and physiology. The usage of these terms is not intended to imply or capture gender identity or social constructs associated with masculinity or femineity.

2.3.2 Study outcomes

All SCr measurements following exposure to RCM were compared to the baseline SCr of the patients. The primary outcome was the development of SCr over time following exposure to RCM compared to baseline SCr. The second outcome was the incidence of CA-AKI defined as a minimum increase of SCr of 50% and/or 26.5 µmol/l compared to baseline SCr, specifically used by the KDIGO (Kidney Disease: Improving Global Outcomes) (“Section 2,” 2012).

2.3.3 Statistical analysis

To minimize the effect of confounders, propensity scores were generated to balance the groups based on their baseline SCr. This method allowed us to create matched pairs of patients with similar propensity scores, ensuring that there was no significant difference in baseline SCr between groups. The development of SCr over time was described using mean value and SD. As the results were normally distributed an ANOVA was used for baseline comparisons and a repeated measures ANOVA was employed to analyze the development of SCr over time. The incidence of CA-AKI was compared between both RCM groups using proportions. Proportions were calculated for each group. A 95% confidence interval (CI) was used to estimate the precision of the proportions and assess the statistical significance of the observed differences. To account for multiple comparisons, the p-values were corrected using the Bonferroni Correction method. Given the analysis involved comparisons of 2 different RCM, as well as 3 different regeneration times, the significance threshold was adjusted to $p < 0.0083$ to maintain an overall Type I error rate of 0.05.

3 Results

3.1 *In vitro* experiments

Figure 1 demonstrates a co-culture of human Fb and HMVEC-D. Angiogenic endothelial cells formed tubes, expanding, branching, and networking with each other to mimic capillary beds. An AI software trained under our supervision performed automated segmentation of the endothelial tubes on light microscope images, furthermore the AI performed a skeletonization of the segmentation and detection of their branches (see Figure 1 b, d, e, f). The AI measured morphometric parameters including “number of branches”, “total tube length” and “average tube diameter”. Results are summarized in Tables 2, 3 & 4. Figure 2 illustrates the temporal development of the three aforementioned morphometric parameters in groups exposed to Iobitridol, Iodixanol, EDTA, Trometamol, and the negative control group.

3.1.1 Morphometric assessment of endothelial tubes after exposure to test agents, depending on exposure times

Limited differences were observed between both exposure times. The co-cultures were exposed to RCM or their supplements, respectively, for 1.5 minutes or 5 minutes. A significant difference in the number of branches was observed between both exposure times, an exposure time of 5 minutes being associated with a lower number of branches in the co-cultures ($p = 0.003$). However, no relationship between the test agent and the exposure time could be observed for this morphometric parameter (p test agent X exposure time = 0.83). No significant difference was observed between exposure times regarding the average tube diameter ($p = 0.86$) and the total tube length ($p = 0.004$).
Attesting to the consistent outcomes observed across both exposure times, it was determined that this variable had minimal impact on the results. Therefore, in subsequent analyses and discussions, exposure time will not be considered as a co-variable, allowing a focused assessment of other variables in our study. Results obtained for both exposure times were aggregated for analysis.

3.1.2 Morphometric assessment of endothelial tubes in negative controls (Figure 3)

The negative control co-cultures were treated under similar conditions as described for the exposed co-cultures and were exposed exclusively to PBS, instead of PBS and a test agent. During the 1st day of regeneration, the total tube length and the number of branches increased. Between the 1st and 3rd day following exposure to PBS, both the total tube length and the number of branches decreased before increasing again until the 7th day. The average tube diameter of the tubes experienced a permanent decrease over regeneration time.

3.1.3 Morphometric assessment of endothelial tubes after exposure to radiological contrast media (Figure 4)

The development of the morphometric parameters over regeneration time after exposure to RCM is shown in Figure 2 a, c, and e.

Both RCM induced a stronger decrease of branches after 3 days of regeneration. The curves exhibited similar trajectories between the RCM-exposed co-cultures and the control co-cultures. The number of branches increased during the 1st day following exposure, decreased between the 1st and 3rd day, before increasing again until the 7th day. After 3 days of regeneration, both RCM had induced a significant stronger decrease of the number of branches per mm^2 compared to control co-cultures ($p < 0.001$). No difference was seen between exposed groups. Directly after exposure and after 1 day and 7 days of regeneration, no significant difference was observed between RCM-exposed and control groups (p-values for no regeneration = 0.75; 1 day = 0.28; 7 days = 0.67).

Both RCM induced a stronger decrease of total tube length after 3 days of regeneration. Directly after exposure to respective RCM, the total tube length did not differ from control ($p = 0.79$). Over regeneration time points the trajectories of the curves for the total tube length between the Iodixanol-exposed co-cultures and the control co-cultures were similar to the trajectories described for the number of branches. Conversely, the Iobitridol-exposed co-cultures exhibited a reduction of the total tube length already during the 1st day following exposure, compared to control. However, the difference in total tube length between both RCM and the control groups was not significant at this regeneration time point ($p = 0.01$). Between the 1st and 3rd day of regeneration, both RCM had induced a significantly stronger decrease in total tube length compared to control group ($p < 0.001$), no difference was seen between both RCM. After 7 days of regeneration the total tube length had increased in all groups, showing no significant difference between RCM-exposed groups and the control group nor between the RCM ($p = 0.84$).

Development of the average tube diameter was not significant. The application of RCM did not induce any significant effect on the average tube diameter of the tubes across all regeneration time points compared to control co-cultures (p-values for no regeneration = 0.028; 1 day = 0.15; 3 days = 0.25; 7 days = 0.096). Between the 1st and 3rd day of regeneration, a slight increase in the average tube diameter was observed in the co-cultures exposed to Iobitridol and Iodixanol, which was not observed in the control co-cultures. However, this difference in the development of the average tube diameter over time was not significant (p test agent X regeneration time = 0.95).

3.1.4 Morphometric assessment of endothelial tubes after exposure to supplements of radiological contrast media (Figure 5)

EDTA induced changes after 3 days of regeneration, Trometamol induced none. The development of the morphometric parameters over regeneration time after exposure to EDTA and Trometamol, respectively are shown in Figure 2 b, d and f. For the total tube length and the number of branches, an increase during the 1st day, followed by a decrease between the 1st and the 3rd day, and a further increase until the 7th day following exposure was observed. These trends were similar to the trajectories of the curves described for control and RCM-exposed co-cultures. EDTA induced a stronger decrease in number of branches between the 1st and the 3rd day of regeneration compared to the control group ($p < 0.001$). The decrease induced by EDTA mirrored that induced by both RCM ($p = 0.46$). EDTA did not cause any significant difference in the total tube length and the average tube diameter over regeneration time compared to control co-cultures. Regarding Trometamol, none of the three morphometric parameters differed from control co-culture.

3.2 *In vivo* experiments

3.2.1 Patients' characteristics and propensity score adjustment

A total of 7'628 cardiac catheterization including intra-arterial RCM injection had been performed between January 2010 and October 2022. The patients were referred for cardiac pathologies, with the majority presenting acute coronary syndrome, necessitating cardiac catheterization for therapeutic and/or diagnostic purposes. Among these, 735 cardiac catheterization met the specified exclusion criteria in this study. Furthermore, 160 patients were excluded due to missing data (height, weight, type of RCM and volume of RCM).
Out of the 6733 remaining patients, 3684 patients had received Iobitridol (Xenetix 300®) and, 2778 Iodixanol (Visipaque 320®). 271 patients had received other RCM. For 410 patients of the Iobitridol group and 303 patients of the Iodixanol group, measurements of baseline SCr and on day 1, 3 and 7 following cardiac catheterizations were available. Following propensity score adjustment based on baseline SCr, a total of 410 patients in the Iobitridol group and 226 in the Iodixanol group were matched to obtain similar baseline SCr between both groups ($p = 0.89$).
As seen in Table 5, there was a significant difference in the distribution of women and men between both groups. Regarding the remaining demographic, clinical and protocol related data, both groups were similar.

3.2.2 Effects of intra-arterial radiological contrast media application on serum creatinine concentration

A significant increase in SCr was observed 3 days after exposure to RCM. The SCr was measured 1 day, 3 days and 7 days following intra-arterial injection of RCM and compared to baseline SCr. The average baseline SCr was 125.9 µmol/l ± 70.3 in the Iodixanol group and 124.80 µmol/l ± 60.99 in the Iobitridol group. Over the 3 measurement time points, the SCr did not vary between groups ($p = 0.63$) (Figure 6). However, the development of SCr following RCM injection was significantly different depending on the day of measurement ($p < 0.001$). The 3rd day following exposure to RCM was associated with a significant increase in SCr of 6.04 µmol/l ± 70.81 in the Iobitridol group and of 3.64 µmol/l ± 61.14 in the Iodixanol group, compared to baseline SCr. After 1 day, the SCr showed a decrease of 1.55 µmol/l ± 40.6 in the Iodixanol group and of 4.65 µmol/l ± 40.55 in the Iobitridol group. After 7 days, the SCr had a decrease of 6.04 µmol/l ± 70.81 in the Iobitridol group and 3.64 µmol/l ± 61.14 in the Iodixanol group.

3.2.3 Incidence of contrast-associated acute kidney injury following intra-arterial administration of radiological contrast media

A significant increase in incidence of CA-AKI was observed 3 days after exposure to RCM. The incidence of CA-AKI 1 day, 3 days and 7 days following cardiac catheterization was calculated based on the KDIGO (Kidney Disease: Improving Global Outcomes) guidelines and defined as a minimal increase of 50% and/or 26.5 µmol/l of the SCr of the patients based on their own baseline SCr values ("Section 2," 2012).
The incidence of AKI was significantly higher 3 days after exposure compared to 1 day ($p < 0.001$). After 1 day, CA-AKI occurred in 29 of 226 patients of the Iodixanol group (12.8%; 95% CI: 8.4%, 17.3%) and in 57 of the 410 patients of the Iobitridol group (13.9%; 95% CI: 10.4%, 17.4%) (Figure 7). No significant difference was observed between groups ($p = 0.53$). After 3 days, an increase in the incidence of CA-AKI was seen in both groups, without difference between groups ($p = 0.33$), with 55 of the patients in the Iodixanol group (24.3%; 95% CI: 19.2%, 29.5%) and 86 of the patients in the Iobitridol group (21%; 95% CI: 17.2%, 24.9%) developing CA-AKI. The incidence of CA-AKI again decreased 7 days after procedure to 45 patients in the Iodixanol group (19.9%; 95% CI: 14.9%, 24.8%) and 68 patients in the Iobitridol group (16.6%; 95% CI: 13.3%, 19.9%). No difference was observed between groups ($p = 0.29$). It is worth mentioning that some of the patients had a doubling of their SCr. After 1 day, 1 of 226 patients of the Iodixanol group (0.4%; 95% CI: 0%, 2.4%) and 6 of 410 patients of the Iobitridol group (1.5%; 95% CI: 0.5%, 3.2%) had their SCr more than doubled based on their own baseline. After 3 days, this was observed in 6 patients of the Iodixanol group (2.7%; 95% CI: 1%, 5.7%) and 18 patients of the Iobitridol group (4.4%; 95% CI: 2.6%, 6.8%). After 7 days, 5 patients of the Iodixanol group (2.2%; 95% CI: 0.7%, 5.1%) and 20 patients of the Iobitridol group (4.8%; 95% CI: 3%, 7.4%) still had their SCr doubled. Over regeneration time, no significant difference was

observed between groups. It is important to note that none of the patients required a renal replacement therapy.

4 Discussion

4.1 Study design

In this study, we assessed the effect of two RCM *in vitro*, using co-cultures of Fb/HMVEC-D forming endothelial capillary beds. To assess the effect of the RCM, we performed a static exposure of the co-cultures to Iobitridol or Iodixanol. This approach allows us to mimic the exposure time and dose of RCM under which renal exposure following intra-arterial administration of RCM commonly occurs (Franke et al., 2008; Kaessmeyer et al., 2017a; Kutt et al., 1963). We morphometrically quantified the changes of the capillary beds after exposure and over regeneration time points. The regeneration time points were based on the definition of CA-AKI as an increase in SCr 48 to 72 hours following RCM administration (Mehran and Nikolsky, 2006).
Regarding the cell culture protocol, we opted to start with a protocol that is well established in research on microvasculature, to ensure that our results would not be distorted by protocol errors. In previous studies on the effect of RCM on microvasculature, most of the endothelial cells used are macrovascular Human umbilical vein endothelial cells, cultured as 2D monolayers (HUVEC; (Boehme et al., 2002; Franke et al., 2012)) or capillary forming HMVEC-D, in complex 3D *in vitro* models (Kaessmeyer et al., 2017a). We chose to use microvascular endothelial cells over macrovascular endothelial cells for their high plasticity and adaptability to organ microenvironment (Augustin and Koh, 2017; Rafii et al., 2016). The protocol performed in our study to generate our co-cultures was similar to the protocol described by Kaessmeyer et al (Kaessmeyer et al., 2017b). In their study, they report the importance of Fb in activating endothelial angiogenesis. Furthermore, their angiogenic potency was characterized in detail, using light microscope and transmission electron microscope, they observed and described the development of tubular structures, built up by the connection of elongating endothelial cells through tight junctions. Additionally, they observed a lumen formation within the endothelial cells. Moreover, Kaessmeyer et al. highlighted the similarities of their *in vitro* construct to the topography of histological tissue sections. Using a similar protocol, we believe that the same phenomenon occurred in our co-cultures, forming lumenized capillary beds. The morphometrically analyzed parameters of the capillary beds were the total tube length, the average tube diameter, and the number of branches. These parameters were already used by Kaessmeyer et al. in their studies evaluating morphological changes in microvasculature *in vitro* (Kaessmeyer et al., 2017b). Undoubtedly, the use of Human kidney microvasculature endothelial cells (HKMEC) would have been ideal in our study. However, little to no studies report the use of renal microvasculature endothelial cells (Thompson et al., 2023; Zhang et al., 2020). This is due to the lack of commercially available cells. Furthermore, recent studies describe protocols for isolation and culture of HKMEC, but these projects are still to be optimized. Indeed, authors report challenges such as variability in the purity of HKMEC, difficulties in maintaining these cells *in vitro*, and a loss of their phenotypes (Thompson et al., 2023).

4.2 *In vitro* experiments

The key and noteworthy finding of our *in vitro* experiment is the more pronounced changes observed 3 days after exposure to Iobitridol and Iodixanol. Consistent and synchronized trajectories of the curves of the parameters «number of branches» and «total tube length» over regeneration time were observed in all exposed co-cultures as well as in the negative control co-cultures. However, the magnitude of the changes was different between groups, with a stronger decrease of both above mentioned parameters 3 days after exposure to RCM. Furthermore, Iobitridol induced slightly more changes than Iodixanol, with a decrease in the total tube length already observed after 1 day of regeneration. Given that Iobitridol is a LOCM, these results align with the studies carried out by Franke et al. (Franke et al., 2013, 2011), that suggested that LOCM tends to induce more pronounced changes *in vitro* compared to IOCM. Studies report that this phenomenon can be explained by the higher osmolality of LOCM, inducing a stronger direct toxicity on the endothelial and epithelial cells (Cho and Ko, 2022). Morphologically, Franke et al. reported a decrease of cell-cell contacts with an increase of denuded subendothelial matrix areas visible on microscope images of co-cultures of endothelial cells and Fb exposed to RCM (Franke et al., 2011, 2008). We postulate that these alterations, can be manifested in our co-cultures as a reduction in both the total tube length and the number of branches, as observed through our analysis on light microscope images.

The RCM used in this study were commercial formulations, also containing EDTA and Trometamol. EDTA is used as a chelating agent to maintain sample purity. Cases of acute renal failure following administration of EDTA have been reported, yet its potential nephrotoxicity is still discussed (Barton, 2014). Trometamol is a buffer substance present in pharmaceuticals such as RCM, midazolam, or non-steroidal anti-inflammatory drugs. While, allergic reactions to gadolinium-based contrast media containing Trometamol have been reported (Lukawska et al., 2019), little is known about its effect on endothelial cells and renal function (Motz et al., 2006). To help determine to what extent these supplements might affect endothelial cells, in our study, co-cultures were exposed to either EDTA or Trometamol with the same protocols as performed for the exposure to commercial RCM. After 3 days of regeneration, EDTA had induced changes similar to those observed in the co-cultures exposed to RCM. In contrast, Trometamol did not induce any significant changes compared to negative control co-cultures. Considering these observations, it is now to be determined if the changes on microvasculature following exposure to the commercial formulations of RCM are attributable exclusively to EDTA or also to the contrast agent itself.
The co-cultures were exposed to the test agents during two different exposure times. In contrast to prior studies (Franke et al., 2011; Kaessmeyer et al., 2017a), only minor differences were noted in the exposed groups between exposure times. Prolonged exposure time was consistently associated with a reduced number of branches.
In this study, we observed changes in the control co-cultures exposed exclusively to PBS, which partly mirrored the changes observed in the co-cultures exposed to PBS and a test agent. This finding raises the possibility that the PBS or elements inherent to our co-culture protocol might have impacted the capillary beds. While PBS is commonly used in *in vitro* studies, its potential influence on our results cannot be overlooked. It has been highlighted that inert substances such as PBS could induce changes in cell cultures (Chen et al., 2017). Therefore, comparison of our *in vitro* results with negative control co-cultures was important, allowing us to distinguish between the specific effects induced by the test agents and natural variations or experimental artifacts.

4.3 *In vivo* experiments

While multiple studies have assessed the effect of RCM on microvasculature *in vitro* (Franke et al., 2013, 2011; Kaessmeyer et al., 2017a; Ramponi et al., 2007; Zhang et al., 2000), translating these results to clinical practice remains challenging. Only few studies report the clinical significance of their *in vitro* findings (Franke et al., 2011; Heyman, 2005). Studies of intra-arterial RCM administration lack of negative control groups of patients undergoing similar procedures without RCM exposure (McDonald and McDonald, 2023). However, intravenous administration of iodinated RCM (Schönenberger et al., 2019; Wu et al., 2022) or intra-arterial administration of carbon dioxide as a negative contrast agent have both shown lower incidence of CA-AKI than intra-arterial injection of iodinated RCM (Ghumman et al., 2017; Wagner et al., 2022). This highlights the need for a better understanding on the pathogenesis of CA-AKI following intra-arterial RCM administration. Aiming to assess the clinical relevance of the results of our *in vitro* experiments, we performed a retrospective study on the renal function of patients who underwent cardiac catheterization including an intra-arterial administration of Iobitridol or Iodixanol. We opted to analyze SCr in patients who received intra-arterial administration of RCM instead of intravenous administration, guided by consistent research findings demonstrating a higher CA-AKI incidence after intra-arterial exposure to RCM. In our study, the principal outcome of the *in vivo* experiment was the increase in SCr compared to baseline SCr accompanied by an increased incidence of CA-AKI 3 days after exposure. This finding is consistent with the definition of CA-AKI, also known as contrast-induced nephropathy, characterized by a rise in renal values 48 to 72 hours after exposure to RCM (Mehran and Nikolsky, 2006). Furthermore, no difference was observed between both RCM in the development of SCr neither in the incidence of CA-AKI over regeneration time. Our study did not assess patients beyond 7 days, which limited our ability to determine the presence of any potential sequelae or long-term effects.

4.4 Qualitative comparison of *in vitro* and *in vivo* experiments and clinical relevance

Based on the findings of Kaessmeyer et al (Kaessmeyer et al., 2017b), highlighting similarities in the construct of their co-cultures of Fb and HMVEC-D and the topography of histological tissue sections, we qualitatively compared the results of our *in vitro* and *in vivo* experiments. To properly compare both exposure types, care was taken to perform measurements at similar regeneration times, i.e., 1 day, 3 days and 7 days following exposure. No SCr values from the *in vivo* experiments were available directly after exposure to RCM.

Our hypothesis posits that a qualitative comparison of both outcomes will allow identification of differences and similarities between *in vitro* and *in vivo* exposures. Interestingly, inversely proportional curves were observed between the development of SCr in patients and the parameters “number of branches” and “total tube length” of the capillary beds in co-cultures. Indeed, between the 1st and 3rd day of regeneration a decrease in morphometric parameters was observed *in vitro,* while the SCr of post-cardiac catheterization patients increased. An opposite phenomenon was visible between the 3rd and the 7th day following exposure, where an increase in morphometric parameters together with a decrease in SCr in patients was observed. The decrease in morphometric parameters could be interpreted as indicative of endothelial damage. We hypothesize that these observed damages might mirror *in vivo* occurrences, potentially inducing renal hypoxia. In a clinical context, this could manifest as a loss of renal function, translated as an increased SCr in the patients. *In vitro*, Iobitridol had induced more changes than Iodixanol. This difference was not observed *in vivo*. Literature known to us previously addressed the phenomenon of *in vitro* studies demonstrating more pronounced disparities between RCM than those observed *in vivo* (Zhao et al., 2011). As mentioned above, EDTA alone induced changes on endothelial cells *in vitro*, similar to those observed after exposure to commercial RCM. However, it is important to note that we did not assess its impact *in vivo*, as we did for the RCM. Therefore, we cannot extrapolate our findings regarding EDTA to physiological conditions.
As mentioned above, it remains difficult to determine if RCM are the primary cause for CA-AKI following intra-arterial administration. These doubts risk taking consideration on the choice of RCM and protocol at the expense of diagnostic and treatment quality. Determining if RCM is the primary cause in CA-AKI following intra-arterial administration will guide decisions on the necessity for specific care of at-risk patients. The preliminary findings of our *in vitro* experiment suggest that we cannot fully exclude RCM as a primary cause of CA-AKI following intra-arterial administration. Therefore, protocol and prophylactic measures should still be adapted to ensure patients safety.

4.5 Limitations

Our study has certain limitations. Foremost, the measurements performed in our *in vitro* study were achieved by an AI software. This allowed us to include a high number of light microscope images. While the parameters “total tube length” and “number of branches” correlated well with manual measurements, the parameter “average tube diameter” showed poor correlation. This highlights the need for additional investigation on the accuracy of the AI, and if necessary, further training of the AI. Regarding our *in vivo* study, the results are not entirely attributable to the administration of RCM. First, due to the challenges to form a sufficiently large group of patients with similar conditions who have undergone cardiac catheterization without the administration of RCM, our *in vivo* experiment lacked a negative control group. Furthermore, demographic and risk factors (McCullough et al., 2021), such as Diabetes mellitus, congestive heart failure or chronic kidney disease (Neyra et al., 2013) have not been considered to evaluate their potential interaction with the effect of RCM on renal function. It would be of particular interest to take these factors into account, as patients with cardiovascular diseases present a higher incidence of the above-mentioned comorbidities (Timmis et al., 2022). This is particularly noteworthy, considering that patients for whom SCr values were available 1 day, 3 days and 7 days after the procedure were likely individuals who had been hospitalized due to complications that could directly have influenced the SCr.
Due to the retrospective character of our *in vivo* study, only routinely measured values such as SCr were available. It is important to note that this value is also influenced by other factors such as the hydration statues of the patients. Furthermore, SCr is used in the evaluation of the renal function and is not representative of cell damage *per se*. Consequently, this value could not be measured in our *in vitro* experiment. The outcomes of the *in vitro* exposure of our study focused on morphometric parameters of capillary beds. Our hypothesis suggests that conducting a qualitative comparison of both outcomes could facilitate identification of disparities and similarities between *in vitro* and *in vivo* methods. The changes observed *in vitro* might be linked with the loss of renal function in patients. To validate this statement, it is essential to measure comparable biomarkers (Franke et al., 2013; Mamoulakis et al., 2019; McCullough et al., 2013; Ramponi et al., 2007; Ren et al., 2017; Zhang et al., 2000), to quantify cellular damages, viability and regeneration both *in vitro* and *in vivo*, and statistically infirm or confirm our theory that the changes observed on the morphology of the endothelial cells *in vitro* could have occurred *in vivo* and lead to the increase of SCr.

5 Conclusion

In conclusion, this study revealed promising similarities between our *in vitro* and *in vivo* experiments, both demonstrating alterations of capillary beds *in vitro* and changes in SCr in post-cardiac catheterization patients three days after exposure to RCM.
Further increase of the complexity of co-cultures by implementing endothelial cells and renal epithelial cells in organ microchip models could allow closer reproduction of the internal structure and microenvironment of a kidney (Roye et al., 2021). This would also involve the induction of a dynamic flow of the test agent through the endothelial structures, together with a progressively decreasing concentration over exposure time to reproduce real-life conditions. Further development of our *in vitro* model to replicate kidney conditions, combined with the use of AI software to increase sample sizes, would allow to bridge the gap between *in vitro* studies on the effects of RCM but also other pharmaceuticals, and their clinical outcomes. This advancement promises meaningful progress in patient care while reducing the need for animal and human research.

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Authors' contributions: SK, UG & JP conceived the idea of the study and contributed to the study design. IM conducted co-cultures for the *in vitro* experiments, IM and JR captured light microscope images, and contributed to the experimental setup. UG provided the database of cardiac catheterization patients for *in vivo* experiments, enhancing the study's clinical relevance. MM trained artificial intelligence algorithms for analyzing light microscope images, conducted statistical analyses for both *in vitro* and *in vivo* experiments, interpreted the results, and drafted the manuscript. GS assisted with statistical analyses, providing valuable expertise in data interpretation. SK, JR & BD contributed to the manuscript by providing critical revisions and important intellectual content. Throughout the project, all authors actively contributed with their expertise and knowledge, enriching the study's overall quality and scope.

All authors have read and approved the final manuscript.

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7 Figures captions

- Figure 1. Representative image of co-cultured Fb and HMVEC-D forming capillary beds. The endothelial cells are labelled with anti-CD31 (a). Same image with segmentation of the tubes by the AI (b, blue). The image (a) was zoomed to illustrate the precision of the AI software before (c) and, after segmentation (d, blue), skeletonization (e & f, green) and highlight of the branches (f, red dots) (phase contrast microscopy).
    - Colored, 1.5 column size

- Figure 2. Temporal development of the morphometric parameters “number of branches” (a, d), "total tube length" (b, e) and "average tube diameter" (c, f) over regeneration time points used for quantification of angiogenesis in the co-cultures after exposure to either Iobitridol (a-c), Iodixanol (a-c), Trometamol (d-f) or EDTA (d-f) and in the negative control cultures (a-f). (No reg indicates no regeneration)
    - Black and white, 1.5 column size

- Figure 3. Negative control co-cultures of Fb and HMVEC-D exposed exclusively to PBS. Images captured directly after exposure to PBS (a, e), 1 day (b, f), 3 days (c, g), and 7 days (d, h) after exposure.
    - Colored, 2 column size

- Figure 4. Co-cultures of Fb and HMVEC-D exposed to Iobitridol (a-d) or Iodixanol (e-h). Images captured directly after exposure to RCM (a, e), 1 day (b, f), 3 days (c, g), and 7 days (d, h) after exposure.
    - Colored, 2 column size

- Figure 5. Co-cultures of Fb and HMVEC-D exposed to Trometamol (a-d) or EDTA (e-h). Images captured directly after exposure to supplements (a, e), 1 day (b, f), 3 days (c, g), and 7 days (d, h) after exposure.
    - Colored, 2 column size

- Figure 6. Temporal development of the SCr of patients compared to baseline, 1 day, 3 days and 7 days following cardiac catheterization with either Iobitridol or Iodixanol.
    - Black and white, single column

- Figure 7. Incidence of CA-AKI 1 day, 3 days and 7 days following cardiac catheterization with either Iobitridol or Iodixanol.
    - Black and white, single column

Table 1. Summary of the parameters measured by the AI and used for morphometric quantification of *in vitro* endothelial capillary bed changes.

	Descriptive statistic	Definition
Total tube length	Sum [μm] per mm^2	Total length of the/all tubes
Number of branches	Total per mm^2	Intersection of two or more tubes, or ramification of a new sprout from an existing tube
Average tube diameter	Mean [μm] of 140 random measures	Diameter of the lumen of a tube, from one side to the other, perpendicular to the skeleton, including endothelial lining

Table 2. Number of branches per mm^2 in the negative control co-cultures and in co-cultures exposed to Iobitridol, Iodixanol, EDTA and Trometamol respectively, directly after exposure, 1 day, 3 days and 7 days following exposure. Mean values and SD values are presented.

	Directly after exposure	1 day after exposure	3 days after exposure	7 days after exposure
Iodixanol	18.79 ± 4.87	26.39 ± 12.69	13.22 ± 4.97	47.12 ± 24.31
Iobitridol	19.72 ± 8.33	21.18 ± 9.51	12.54 ± 3.93	39.85 ± 19.94
EDTA	17.42 ± 3.14	27.53 ± 12.21	13.18 ± 3.7	36.88 ± 17.42
Trometamol	17.97 ± 4.98	26.57 ± 11.78	20.23 ± 10.65	44.83 ± 25.14
Control	20.3 ± 4.66	29.42 ± 13.06	25.06 ± 14.74	46.59 ± 21.46

Table 3. Total tube length per mm^2 in the negative control co-cultures and in co-cultures exposed to Iobitridol, Iodixanol, EDTA and Trometamol respectively, directly after exposure, 1 day, 3 days and 7 days following exposure. Mean values and SD values are presented.

	Directly after exposure	1 day after exposure	3 days after exposure	7 days after exposure
Iodixanol	5484.75 ± 735.14	6204.66 ± 1488.91	4198.04 ± 722.43	6991.48 ± 1695.23
Iobitridol	5388.91 ± 795.33	5131.64 ± 1354.42	4239.97 ± 724.27	6714.36 ± 1813.28
EDTA	5176.87 ± 543.8	6175.58 ± 1489.14	4351.31 ± 593.48	6625.13 ± 1494.67
Trometamol	5359.42 ± 589.85	6195.49 ± 1469.04	5174.4 ± 1313.88	6649.73 ± 1644
Control group	5616.2 ± 972.52	6787.09 ± 1154	5729.27 ± 1728.02	6934.73 ± 1681.08

Table 4. Average tube diameter in the negative control co-cultures and in co-cultures exposed to Iobitridol, Iodixanol, EDTA and Trometamol respectively, directly after exposure, 1 day, 3 days and 7 days following exposure. Mean values and SD values are presented.

	Directly after exposure	1 day after exposure	3 days after exposure	7 days after exposure
Iodixanol	10.4079 ± 0.9103	10.0596 ± 0.7116	10.2331 ± 0.992	9.8597 ± 0.4471
Iobitridol	9.9693 ± 0.6436	9.6786 ± 0.6993	9.7485 ± 0.764	9.5953 ± 0.3455
EDTA	11.0788 ± 1.2133	10.1925 ± 0.6834	9.8834 ± 0.9565	9.6262 ± 0.4089
Trometamol	11.1283 ± 1.4001	10.2881 ± 1.0032	10.1784 ± 0.9555	9.9602 ± 0.4818
Control group	10.9264 ± 1.07	10.1128 ± 0.6542	10.0119 ± 0.6788	9.8554 ± 0.1963

Table 5. Patients' demographic, clinical and protocol characteristics for each group (Iobitridol-exposed, and Iodixanol-exposed groups). Mean values, SD values and p-values are presented.

Patient	Iodixanol	Iobitridol	p-value
Total patients, n	226	410	
Male, n (%)	135 (59.7)	298 (72.7)	< 0.05
Female, n (%)	91 (40.3)	112 (27.3)	< 0.05
Age (years)	74 ± 11.71	73 ± 11.95	0.317
Weight (kg)	80.5 ± 16.5	82.4 ± 15.9	0.109
BMI (kg/m^2)	28 ± 5.5	27.9 ± 4.9	0.885
Baseline SCr (µmol/l)	125.9 ± 70.3	124.80 ± 60.99	0.894
Dosis (ml/kg)	1.5 ± 1.07	1.52 ± 0.95	0.062

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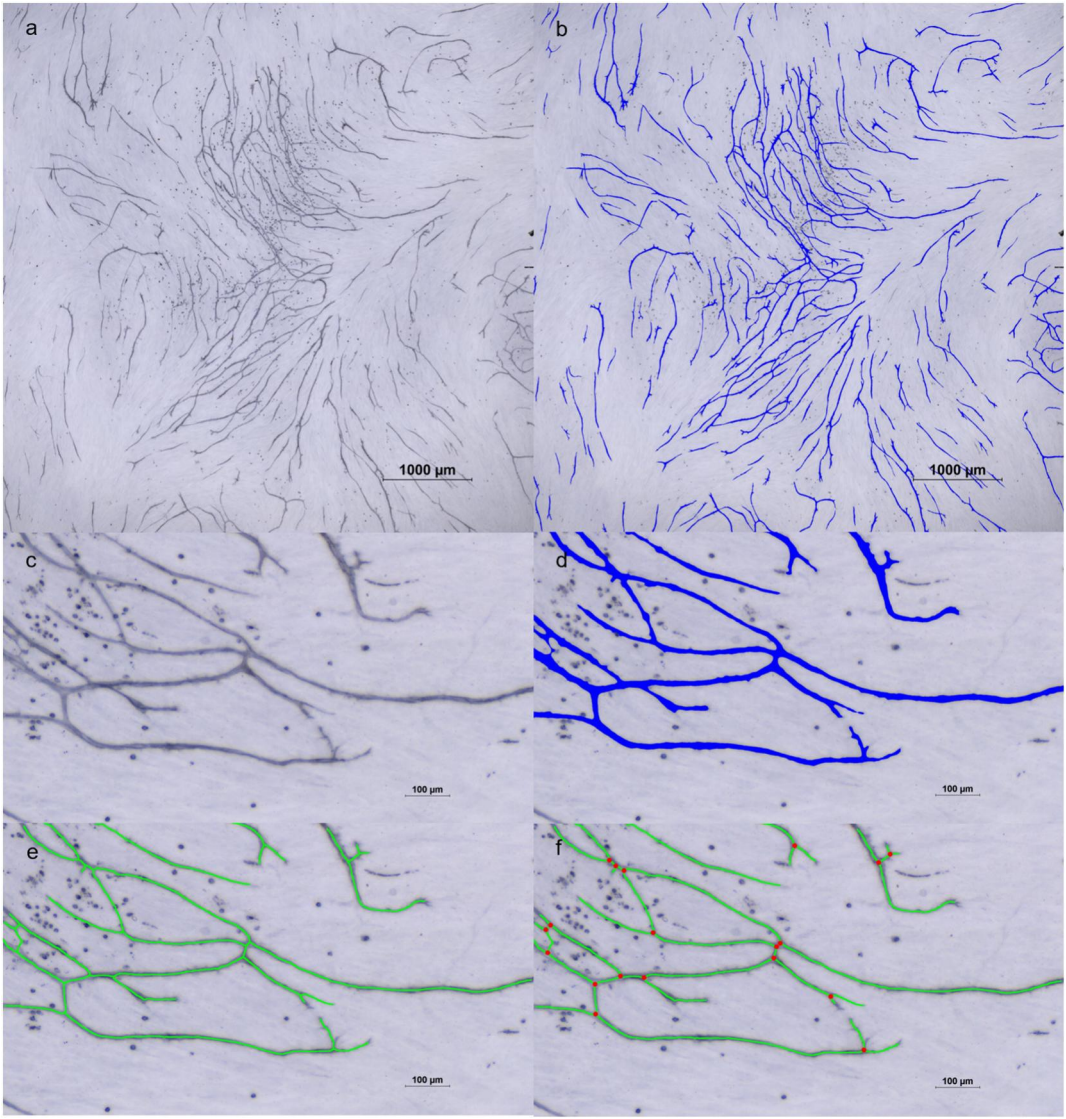

Figure 1

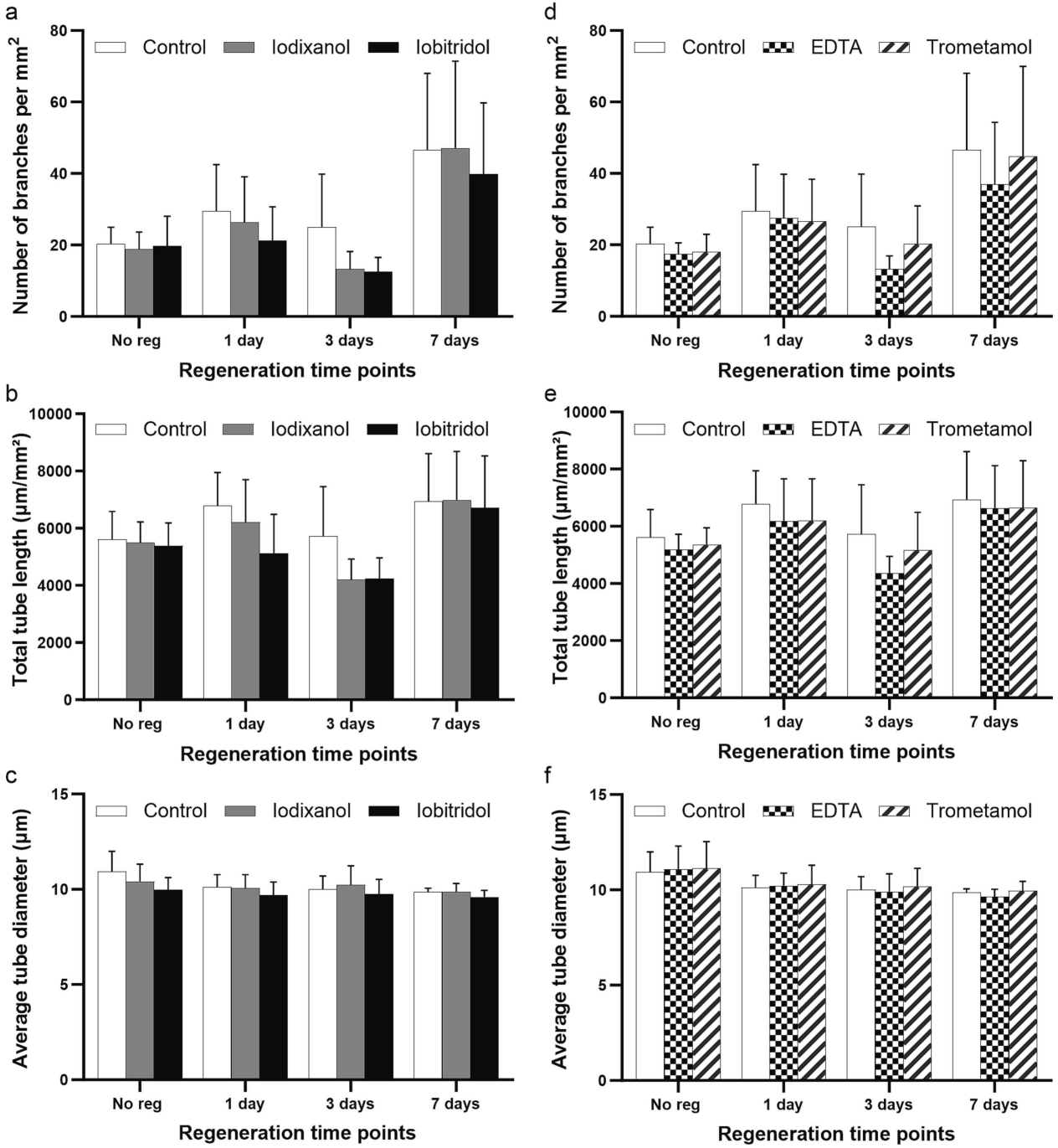
a
Number of branches per mm2
Control
Iodixanol
Iobitridol
No reg
1 day
3 days
7 days
Regeneration time points
d
Number of branches per mm2
Control
EDTA
Trometamol
No reg
1 day
3 days
7 days
Regeneration time points
b
Total tube length (µm/mm²)
Control
Iodixanol
Iobitridol
No reg
1 day
3 days
7 days
Regeneration time points
e
Total tube length (µm/mm²)
Control
EDTA
Trometamol
No reg
1 day
3 days
7 days
Regeneration time points
c
Average tube diameter (µm)
Control
Iodixanol
Iobitridol
No reg
1 day
3 days
7 days
Regeneration time points
f
Average tube diameter (µm)
Control
EDTA
Trometamol
No reg
1 day
3 days
7 days
Regeneration time points

Figure 2

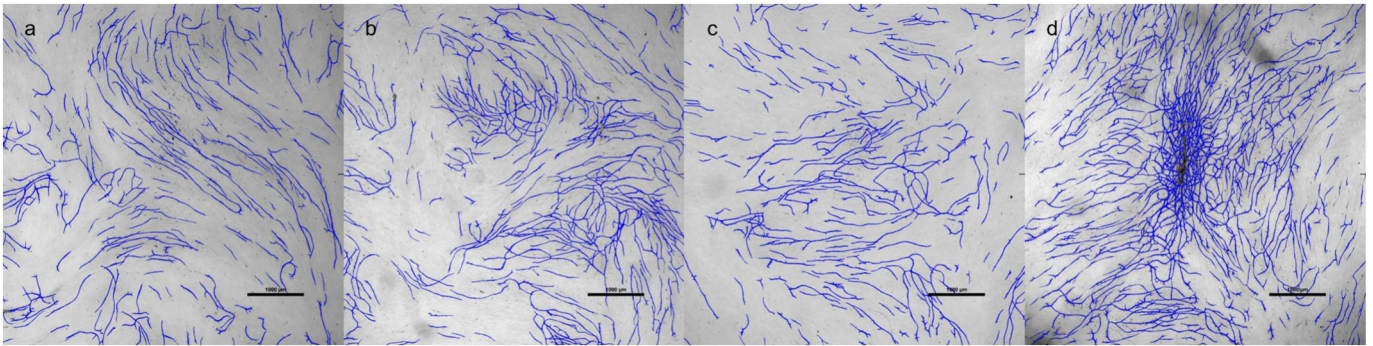

Figure 3

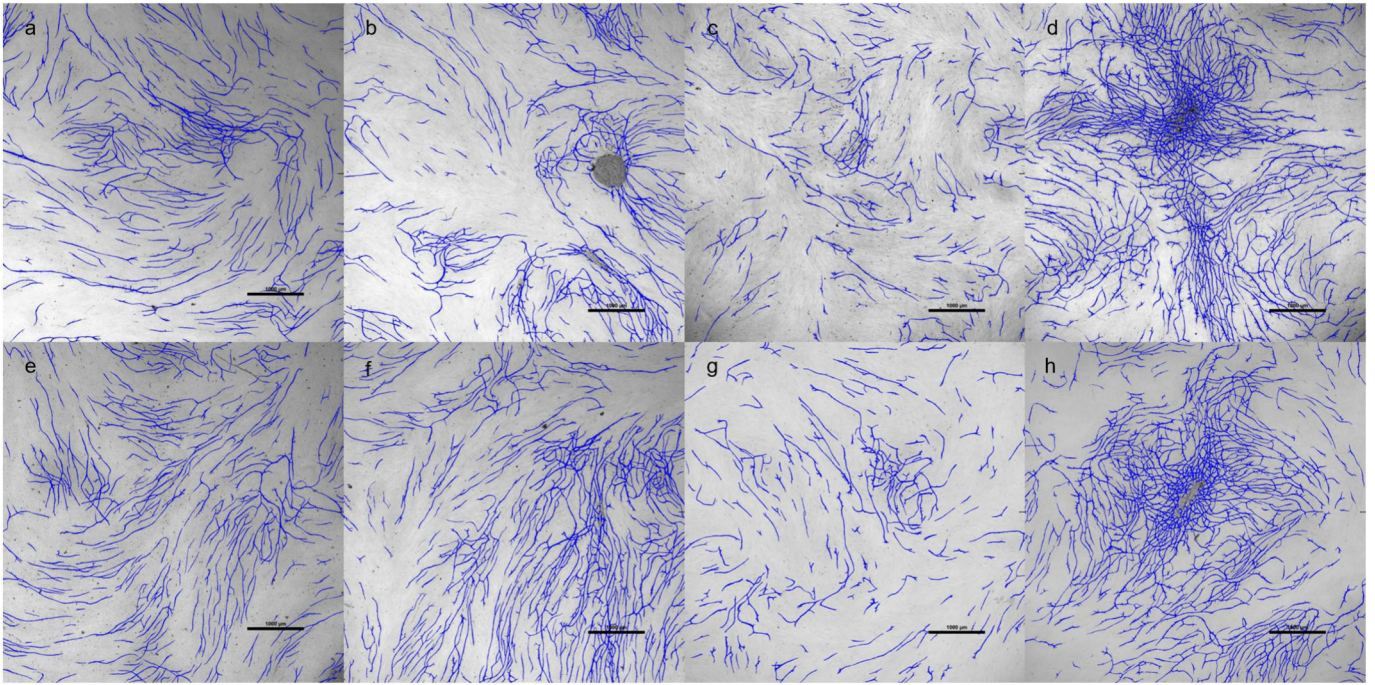

Figure 4

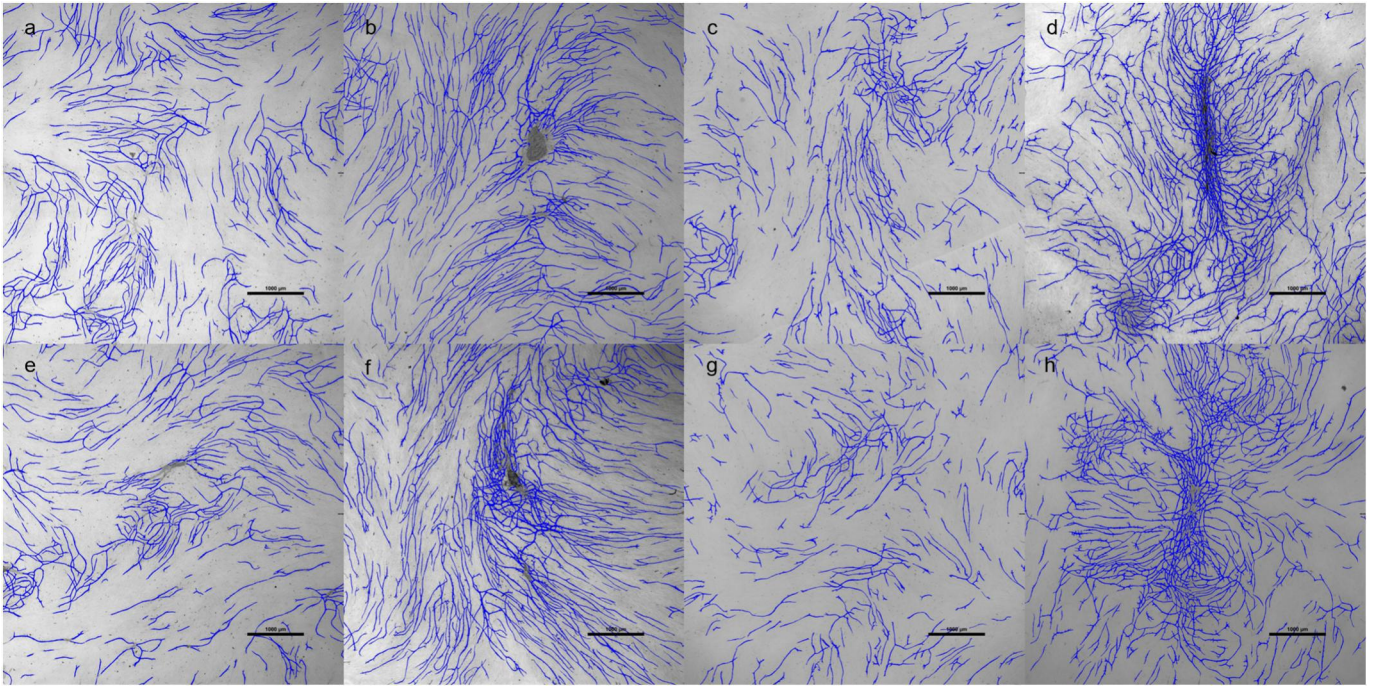

Figure 5

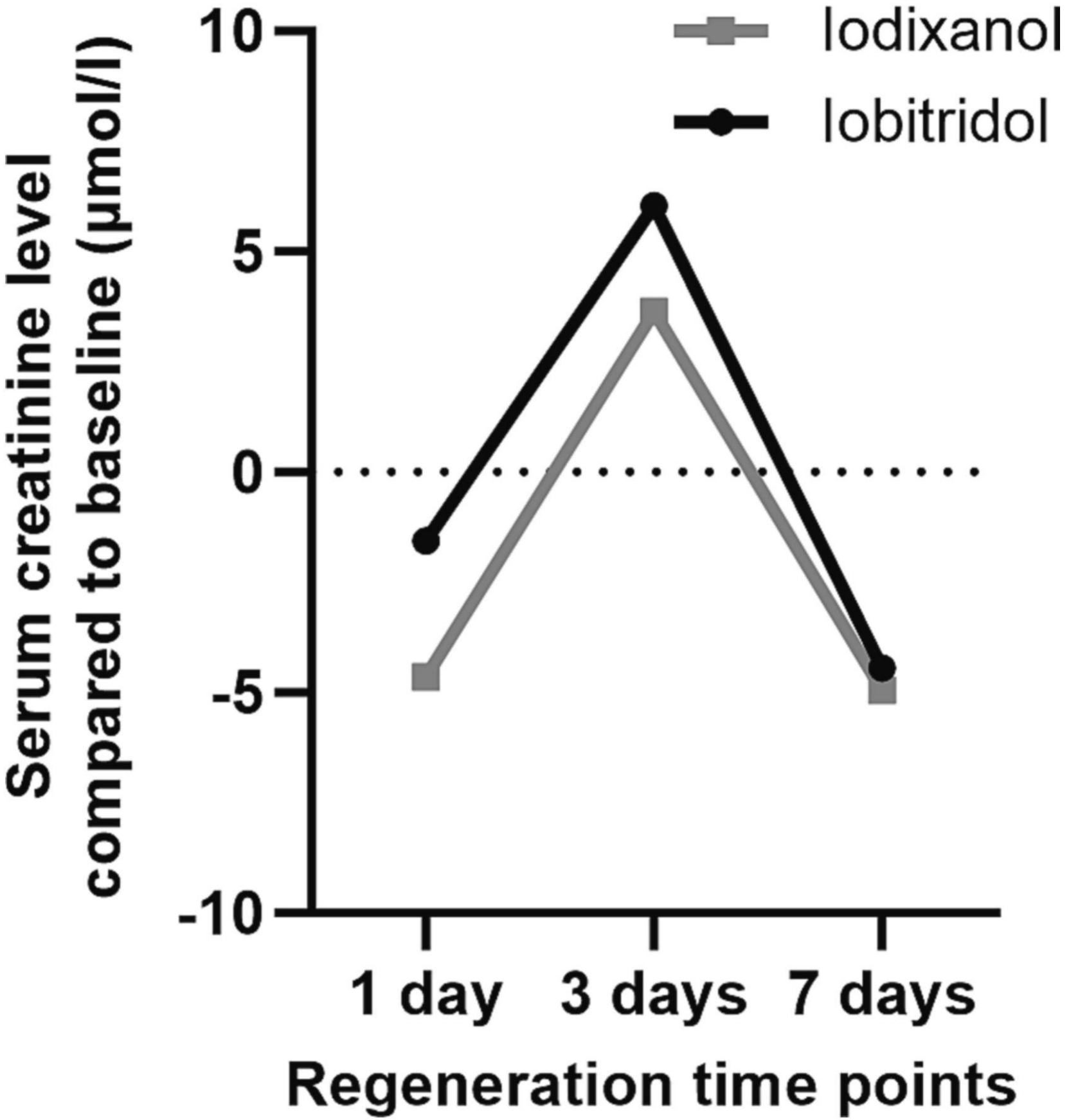
Iodixanol
Iobitridol
10
5
0
-5
-10
Serum creatinine level compared to baseline (µmol/l)
1 day
3 days
7 days
Regeneration time points

Figure 6

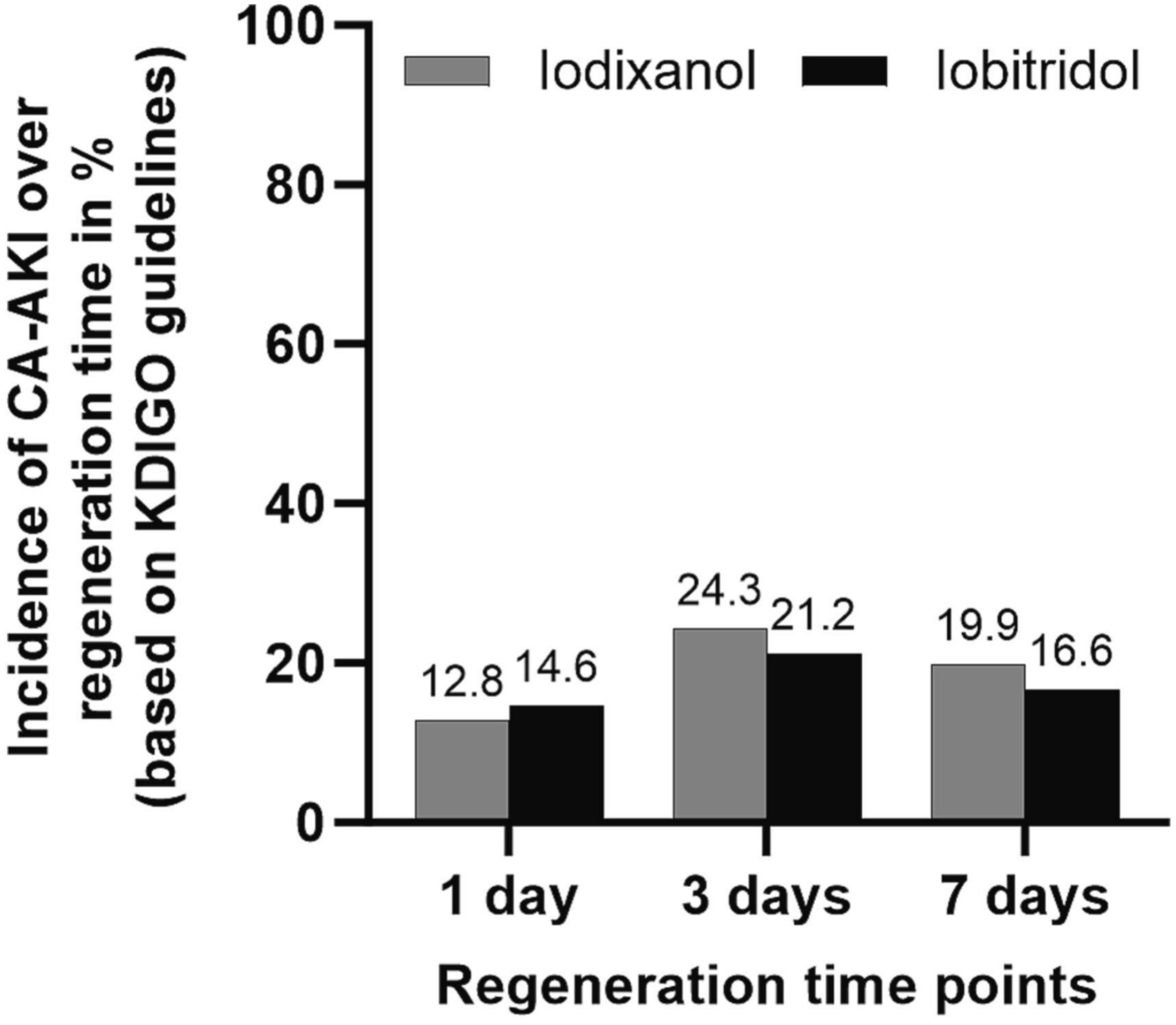
Incidence of CA-AKI over regeneration time in % (based on KDIGO guidelines)
100
80
60
40
20
0
Iodixanol
Iobitridol
12.8
14.6
24.3
21.2
19.9
16.6
1 day
3 days
7 days
Regeneration time points

Figure 7