Original Article

Blood Flow in the Meniscus Can Be Visualized Arthroscopically Using an Intravenous Indocyanine Green Solution Diluted 10× in a Pig Model

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Purpose: To determine the optimal indocyanine green (ICG) dose required for assessing vascularity in the meniscus using ICG fluorescence-guided knee arthroscopy in a pig model. **Methods:** A 3-month-old Japanese esculent female pig was used in this study. Intravenous injections of ICG (25 mg) were administered with 2.0 mL of 5×, 10×, 100×, and 1,000× diluted solutions. An additional experiment was conducted to assess the microvasculature within the meniscus considering the results of the optimal dilution setting. A radial tear was purposely induced in the middle-to-posterior section of the medial meniscus to observe vascularity in the cross-sectioned meniscus; the optimal ICG dilution was administered. **Results:** No fluorescence was detected in the meniscus with solutions diluted by 1,000× and 100×. Fluorescence was visualized at the anterior portion of the synovium and the anterior cruciate ligament using ICG diluted by 10×. Diluting ICG by 5×, contrast enhancement was too intense for observation. Therefore, the 10× diluted solution was considered the optimal setting for knee arthroscopy and observation of the radial tear. No fluorescence was observed in the cross section of the medial meniscus. Arterial hemorrhage was observed by stimulating the fluorescence-dyed synovium adjacent to the tear site. Through the additional waiting time after stimulating the tear site, the hemorrhage inside the meniscus became more intense. **Conclusions:** The optimal dilution and dose setting of ICG for knee arthroscopy was 10× in a 2.0-mL intravenous injection. The meniscus showed no active blood flow, even in the red-red zone. This finding might support the notion that blood flow cannot be initiated, without synovial stimulation, even in vascular areas. **Clinical Relevance:** This study could determine an ICG solution suitable for ICG fluorescence-guided knee arthroscopy. This finding could be valuable in future research focusing on case-specific meniscal vascularization under arthroscopy, particularly applying these findings to human meniscal treatment.

Preserving the meniscus is essential to prevent degenerative arthritis of the knee joint. However, the meniscus is anatomically, mechanically, and biologically conditioned by a special environment, particularly owing to its limited blood supply.

The blood supply to the human meniscus is limited to the outer 10% to 30% of its volume.[1-3] In addition, the vascularity of a meniscal tear may be a potential factor for achieving meniscal repair.[4-6] However, individual and case-specific vascularization and blood supply to the meniscus under arthroscopy are not well understood. Visualizing real-time vascularization of the meniscus based on patient characteristics intraoperatively may enhance the outcomes of meniscal surgery owing to the provision of real-time information for intraoperative decision making (regarding meniscal repair or meniscectomy).

Indocyanine green (ICG), a fluorescent dye, is commonly used as a marker to assess organ blood flow in the field of internal medicine.[7,8] ICG binds rapidly to plasma proteins via intravenous injection. A near-infrared (NIR) light with a wavelength of approximately 750 to 810 nm helps detect fluorescence from the ICG excitation beam.[9-11] Already widely used in clinical practice in surgical fields such as hepatobiliary pancreatic surgery, laparoscopic cancer surgery and sentinel lymph node detection. ICG fluorescence-guided surgery is a modern navigation technique used to identify tissue vascularization and the anatomic structures between tumors and marginal tissues.[8,9,12,13]

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In this study, the fluorescence enhancement properties of ICG were used to visualize the vasculature of the meniscus and its surrounding structures. This study mainly aimed to determine the optimal ICG dose required for assessing vascularity in the meniscus using ICG fluorescence-guided knee arthroscopy in a pig model. The author hypothesized that using an arthroscope with a NIR light as a light source, visualizing the distribution of blood flow in the vascular and avascular regions of the meniscus could be possible. In addition, it was hypothesized that this technique might facilitate real-time identification of blood flow from the synovium to the meniscus, as well as the presence of blood vessels within the meniscus.

Methods

Animal Experiment

The study protocol was approved by the local ethics review board (approval No. K-21-065). A 3-month-old Japanese esculent female pig (crossbreed of Large White, Landrace Dam, and Fast Duroc [LWD]) from Okayama, Japan), weighing 45 kg at the time of this study, was used. Arthroscopy was performed on the right knee of the pig under general anesthesia using the standard lateral infrapatellar approach (Fig 1A). The author used an arthroscope combined with a 1688 Advanced Imaging Modalities 4K Platform camera system and an L11 light source (Stryker, Kalamazoo, MI).

Tourniquets and agents to prevent bleeding were not administered. Ringer solution was used as the annulus fluid as in human surgery to clearly visualize arthroscopy; its flow and pressure were controlled using an arthroscopy pump. The flow pump was adjusted to 0.3 L/min and 0 mm Hg for flow rate and pressure, respectively, to maintain the natural state of blood circulation in the lower extremity as much as possible.

The intra-articular structures in the pig model used resembled those of a human knee joint but were very small (Fig 1 B and C). The observation setting was limited to the medial compartment within the anterior cruciate ligament (ACL).

Preparation and Injection of ICG Solutions

ICG (25 mg, DIAGNOGREEN; Daiichi Sankyo, Tokyo, Japan) was diluted by 5×, 10×, 100×, and 1,000× into ICG solutions for injection (Fig 2). Intravenous injections were performed with 2.0 mL of each diluted solution. These optimal dose tests were set as previously described in recent reports on ICG fluorescence-guided navigation surgery.[8,13-16] A single-bolus intravenous injection of ICG solution was followed by an injection of 10 mL of saline solution.

The experiment began with the administration of lower dilutions of ICG, starting with a dilution of 1,000×. After observation of the complete attenuation of fluorescence from within the joint, after a 15-minute waiting period, administration of the next dilution of ICG (i.e., 100×) was performed. Similarly, after a 15-minute wait after fluorescence attenuation, the next dilution experiment (10× and, finally, 5×) was conducted.

Additional Experiment: Observation of Cross Section of Medial Meniscus

After the optimal dilution setting was determined based on the results of the experiment, additional experiments were performed based on optimal dilution settings. A middle-to-posterior segment transverse

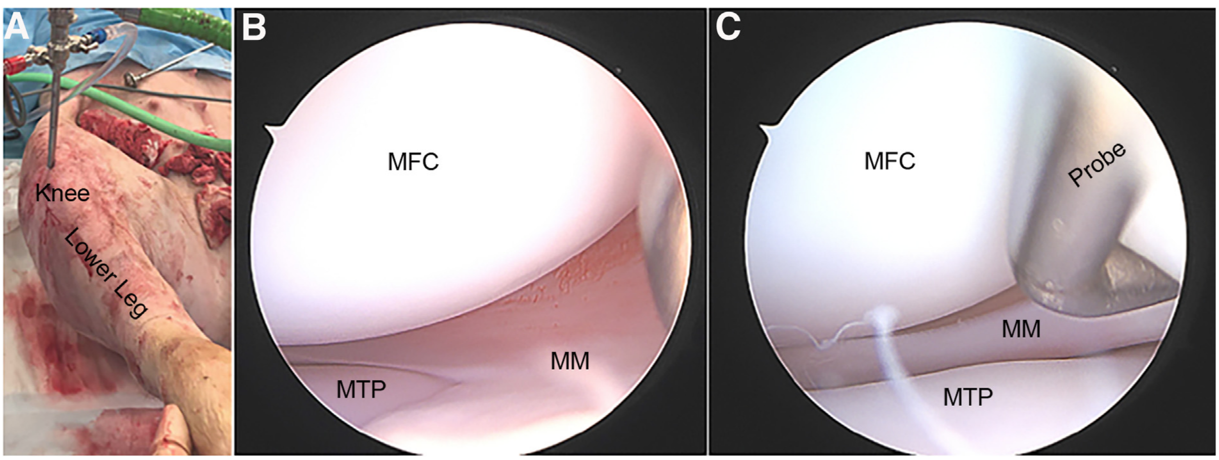

Fig 1. Experimental pig model and arthroscopic observation in SPY Overlay mode of medial compartment with indocyanine green (ICG) dilution setting of 100×. (A) The standard lateral infrapatellar approach is used in the right knee joint for this animal experiment. (B) Standard arthroscopic viewing of medial compartment in experiment with ICG solution diluted by 100×. (C) Probing under medial meniscus. No fluorescence is observed with ICG solution diluted by 100×. (MFC, medial femoral condyle; MM, medial meniscus; MTP, medial tibial plateau.)

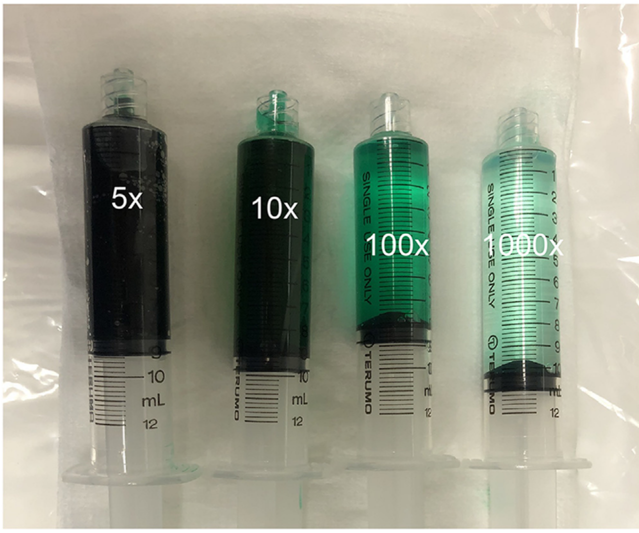

Fig 2. Preparation of indocyanine green (ICG) solutions for injection. ICG (25 mg) is diluted by 5×, 10×, 100×, and 1,000× (from left to right) into ICG solutions for injection.

incision was made in the medial meniscus, resulting in a radial tear to observe vascularity in the inner side of the meniscus from the avascular to vascular zone. A radial tear was intentionally created in the middle-to-posterior segment using a punch and shaver to expose the cross section of the medial meniscus from the white-white zone to the synovial margin of the red-red zone in the meniscus (Fig 3). Administration of the optimal ICG dilution allowed observation of the cross section of the radial tear in the medial meniscus.

Modalities for Observation

ICG fluorescence-guided arthroscopy was used to observe 3 modality patterns: standard arthroscopy, SPY Overlay and SPY Contrast (Stryker). The SPY modes are routinely used in laparoscopic settings to visualize tissue vascularity. The SPY Overlay mode exhibits bright green fluorescence in the standard color arthroscopy mode, whereas the SPY Contrast mode displays bright white ICG fluorescence in the dark monochrome mode, which is the most effective fluorescence detection mode for identifying vascularization.

Results

Observation of Medial Compartment in Experimental Pig Model

Fluorescence could not be detected in either of the 2 SPY modality patterns (SPY Overlay and SPY Contrast modes) with solutions diluted by 1,000× and 100× (Fig 1 B and C). The fluorescent region was visualized at the anterior portion of the synovium and part of the synovium surrounding the ACL using ICG diluted by 10×. No fluorescence was observed at the medial meniscus, even in conspicuous vessels on the medial meniscus, which could be observed using the standard mode (Fig 4 A-C). At the anterior synovium of the ACL, some of the vessels were fluorescent and bright green in SPY Overlay mode (Fig 4D). However, remarkable fluorescence in SPY Contrast mode was observed as bright white light (Fig 4E).

Although ICG was diluted by 5×, the contrast enhancement was too intense for any observation, owing to halation (Fig 5). No fluorescence was observed at the meniscus even when using a higher dilution of ICG. On the basis of the experimental results of the dilution setting, a 10× diluted solution was selected as the optimal solution for knee arthroscopy.

Observation of Cross Section of Medial Meniscus With ICG Solution

Fluorescence was observed in the anterior synovium. The same result was obtained without a cross section of the medial meniscus. No fluorescence was observed at the cross section in either the anterior or posterior region, extending from the white zone to the red zone in the medial meniscus (Fig 6A).

Additionally, by stimulating the fluorescence-dyed synovium adjacent to the tear site using a probe (Fig 6B), bleeding was observed in SPY Overlay mode at the anterior section of the medial meniscus, and arterial hemorrhage was observed in SPY Contrast mode (Fig 6C). Furthermore, rasping was performed at the cross-sectioned tear site, revealing that hemorrhage had

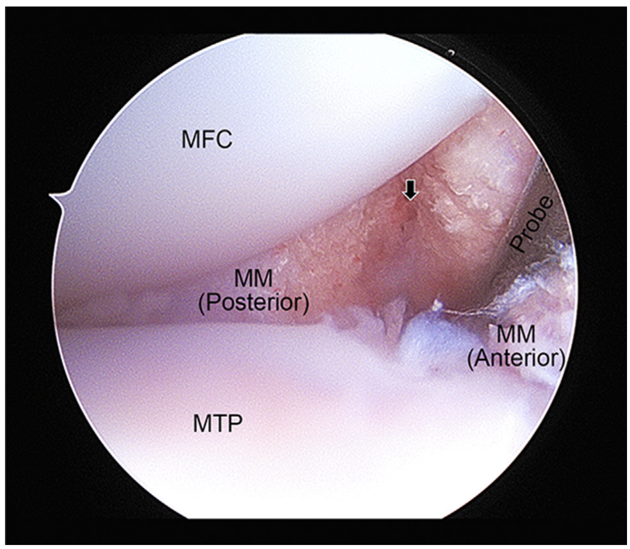

Fig 3. Radial tear made for observation of cross-sectioned medial meniscus. The cross-sectioned radial tears are opened away from each other in 2 opposite directions using a probe. The arrow indicates the center of the synovial margin separated anteriorly and posteriorly at the red-red zone of the meniscus. (MFC, medial femoral condyle; MM, medial meniscus; MTP, medial tibial plateau.)

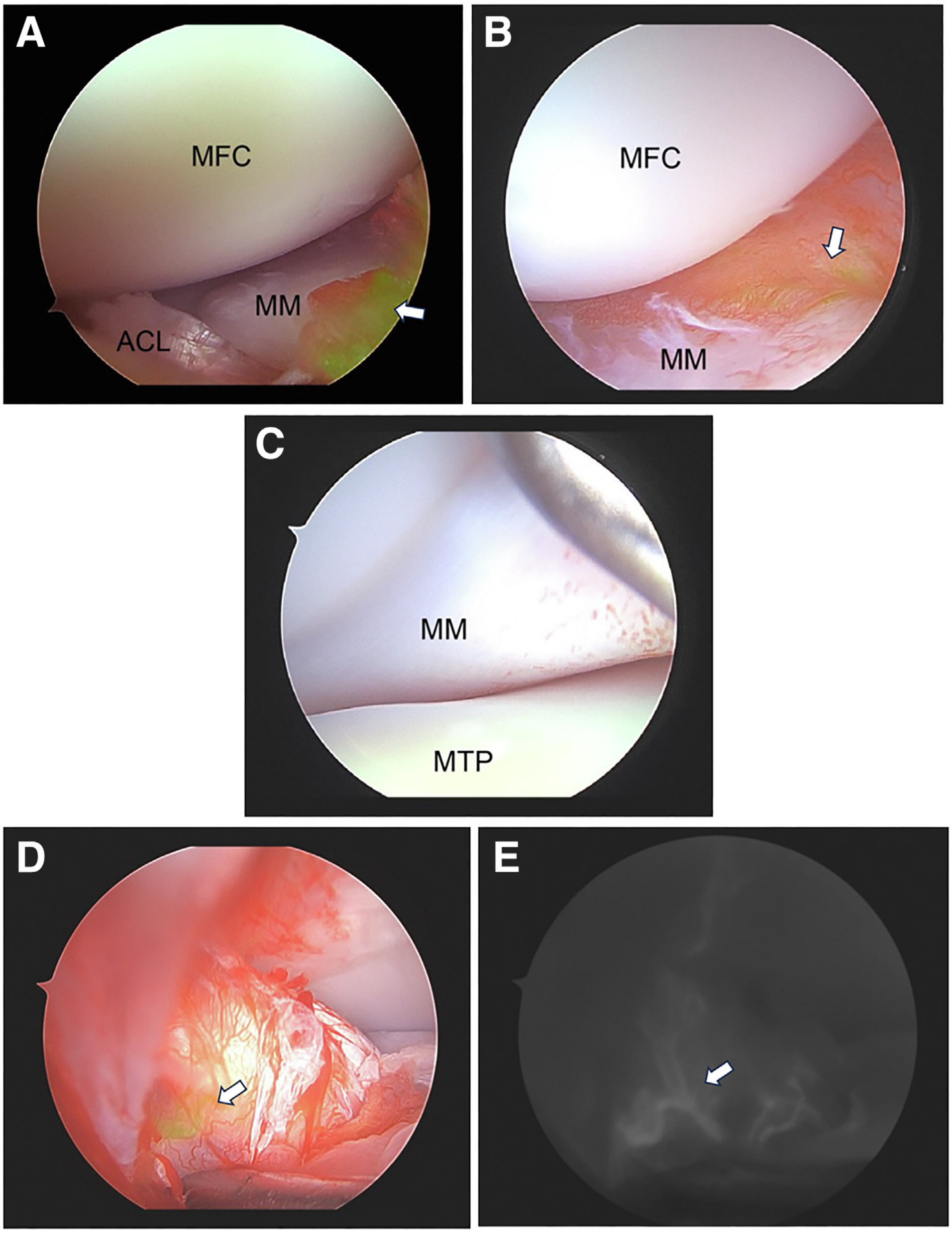

Fig 4. Observation of medial compartment with indocyanine green dilution setting of 10×. (A) Observation of anterior portion of medial compartment. The synovium of the anterior portion of the medial meniscus exhibits fluorescence (arrow). (B) The synovium of the meniscocapsular region of the middle portion of the medial meniscus is light green (arrow). (C) Vessels on the tibial side of the medial meniscus are observed using a probe. No fluorescence is observed at the medial meniscus, even in conspicuous vessels, which could be observed in standard mode. (D) At the anterior cruciate ligament (ACL), parts of the vessels are fluorescent and bright green in SPY Overlay mode (arrow). (E) Remarkable fluorescence of the ACL is observed in SPY Contrast mode as white light emitting brightly (arrow). (MFC, medial femoral condyle; MM, medial meniscus; MTP, medial tibial plateau.)

increased. However, no fluorescence was observed in the posterior section of the medial meniscus (Fig 7A). Through the additional waiting time after stimulation to the tear site, the hemorrhage from the anterior section became more intense as bright light using SPY Contrast mode (Fig 7B). Furthermore, a symmetrical dot hemorrhage was observed in the posterior section (opposite site) of the radial tear of the medial meniscus (Fig 7C, Video 1).

Discussion

The most important finding of this study was that ICG fluorescence-guided arthroscopy could be used for visualizing blood flow in the meniscus using a 10×-diluted ICG solution administered intravenously in a pig model. Additionally, stimulating the fluorescent region of the synovium may activate visualization of blood flow inside the meniscus.

The vascularity of the meniscus is limited, and detailed experimental studies were reported by King[17] in 1936 and Arnoczky and Warren[18] in 1983. Regarding the human meniscus, Arnoczky and Warren[1] examined cadaveric specimens in 1982. They observed that the vascularity of the human meniscus had a limited periphery. The established surgical planning for meniscal repair is grounded in the discoveries of these studies, which propose segmental classifications, such as red-red, red-white, and white-white zones.

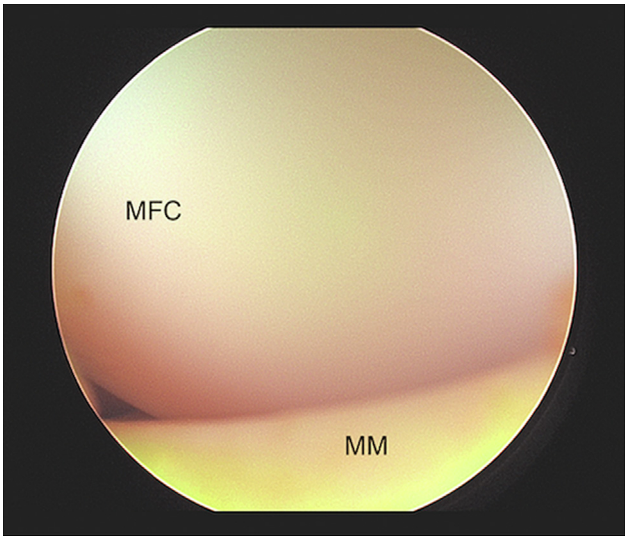

Fig 5. Indocyanine green dilution setting at 5× at medial femoral condyle, which is observed with halation in anterior region. It reveals greenish, blurred, and indistinct findings that are difficult to evaluate accurately. (MFC, medial femoral condyle; MM, medial meniscus.)

Recent findings have led to conflicting opinions regarding the meniscal vasculature, with some studies suggesting its extent is limited and others indicating that it is abundant.[19-22] In a study conducted by Michel et al.[20] in 2021, no blood vessels were reported to be present in the red-white zone, revealing that this finding was consistent with age-related changes. However, Crawford et al.[21] examined the microvascular structure of the meniscus in young adults and concluded that the vascular supply of the medial and lateral menisci was more extensive than previously reported in older specimens; however, the median values were consistent. Moreover, Chahla et al.[22] found that blood vessels were also present in avascular areas in young adults. Recent studies have revealed findings that could contrast with those of other previous reports on the blood supply in the meniscus, revealing divergent outcomes. As a result, the traditional zone classification as a prognostic indicator is becoming increasingly complex and difficult to assess. On the other hand, recent technological advancements have enabled preservation of the meniscus that can now be maintained with greater success in these patients.[5,6,23] Given the increasing popularity of meniscal surgery among a diverse group of patients, surgical planning must consider a variety of patient-specific factors. Furthermore, to enhance surgical outcomes, considering patients' lifestyle habits, such as smoking and alcohol consumption, at the time of surgery is crucial, given that they can

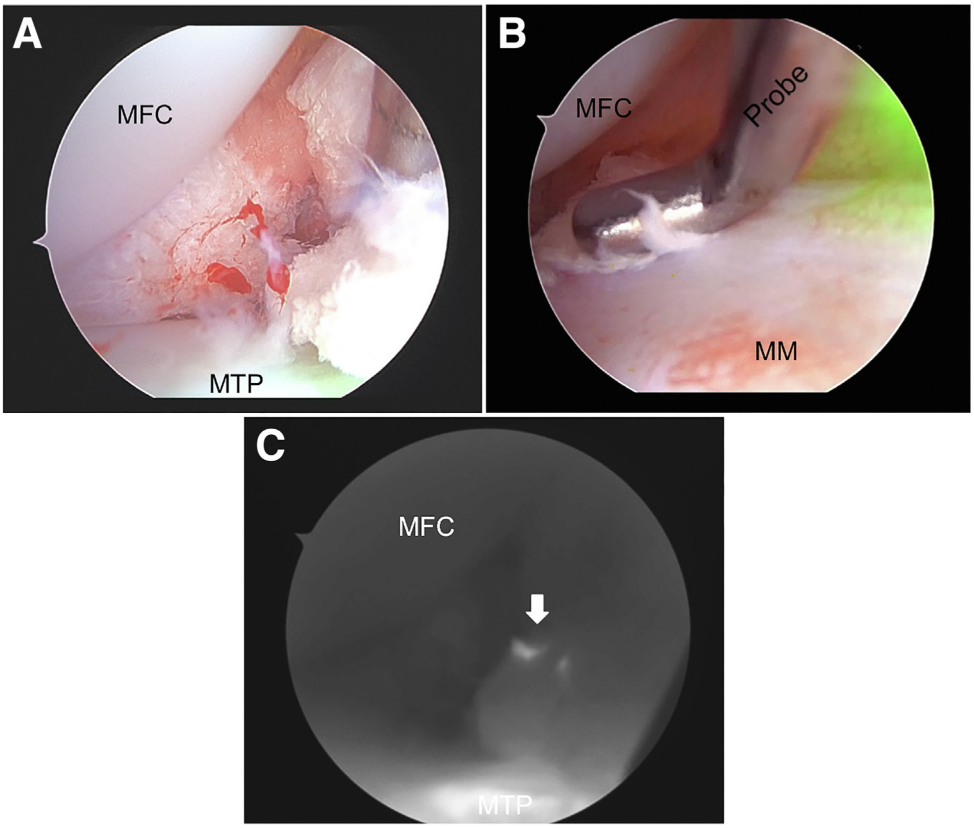

Fig 6. The fluorescence-dyed synovium adjacent to the tear site is pressed using a probe. (A) No fluorescence is observed at the cross-sectioned site. (B) The synovium adjacent to the tear site is pressed. (C) Bleeding is observed on the anterior side of the cross section in SPY Contrast mode as small white dots (arrow). (MFC, medial femoral condyle; MM, medial meniscus; MTP, medial tibial plateau.)

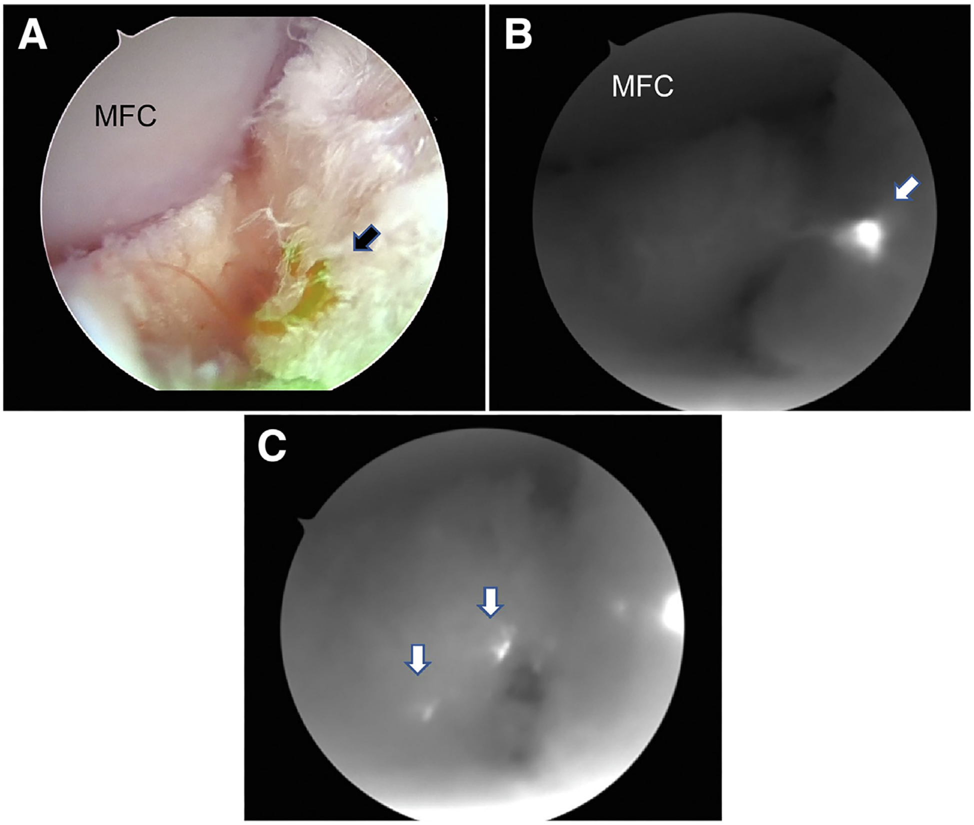

Fig 7. Increased hemorrhage is observed after rasping. (A) Active bleeding is observed after rasping at the anterior site of the cross section in SPY Overlay mode (arrow). (B) SPY Contrast mode reveals bright dots at the anterior site of the cross section (arrow). (C) After the waiting period, symmetrical bright dots are observed at the posterior site (opposite site) of the cross-sectioned medial meniscus (arrows). (MFC, medial femoral condyle.)

significantly affect arteriosclerosis and peripheral circulation.[24-26]

Several questions arise concerning the prognosis of meniscal repair considering the injured area of the meniscus and whether the latest technology could achieve a high success rate in any patient. It is important to highlight that meniscal repair may still result in failure, even when the surgeon makes the same level of effort as in cases of successful outcomes. However, visualizing the vascularity of the meniscus may aid in predicting the prognosis in each case.

ICG fluorescence-guided surgery is an optical imaging guidance procedure that is used during surgery. In 1987, Sugimoto et al.[27] reported the laparoscopic findings of hepatic necrosis after intravenous ICG injection. In 2009, Ishizawa et al.[14] reported the first instance of ICG fluorescence-guided cholecystectomy, and subsequently, there has been increasing interest in optical imaging guidance procedures using endoscopy with a NIR light source for accurate evaluation of the target organ.[8,12,13] Although there have been some limited applications of ICG in orthopaedics, the use of this technique has not yet been widely established as a clinical application.[28-30]

In this study, the active vascularity of the meniscus was very poor in a pig model using ICG fluorescence-guided arthroscopy. Overall, the anterior meniscal portion of the synovium was more fluorescent than were other structures. These findings support the description of Arnoczky and Warren.[1] In an additional experiment involving a cross-sectioned meniscus that included the vascular zone, although the tear was a fresh injury, there was no visualized hemorrhage. However, under stimulation of synovial conditions, blood flow could be visualized inside the meniscus.

Additionally, dot hemorrhage was observed after some time. Visualization of the dot hemorrhage on both sides of the tear site was not obtained instantly but required a waiting period of approximately 10 minutes in this experiment. It was observed that the duration from the stimulation of the synovium to the activation of blood perfusion was longer and the blood flow velocity was slower than anticipated. The appearance of the blood flow inside the meniscus and its moment were captured in this study (Video 1).

The ability to detect real-time blood perfusion is the greatest advantage of ICG fluorescence-guided surgery. Target tissues emit fluorescence with a NIR light containing ICG. Therefore, plasma proteins are located at the fluorescent site, and the site may have the potential to promote healing.

Although this study yielded some noteworthy findings, there were also some questionable findings that require further investigation. Specifically, the visually confirmed blood vessels may not always exhibit fluorescence (Fig 4). These conspicuous blood vessels at the ACL are a particularly notable finding (Fig 4 D and E). However, their interpretation is challenging and may be influenced by the velocity and/or activity of blood perfusion.

Acknowledging that the hemodynamics of the meniscus remains to be elucidated is imperative. In addition, it is possible that conspicuous blood vessels may not always display robust circulation. In certain instances, they may become congested or obstructed by weight bearing within the knee joint, which is consistent with previous investigations into the anatomy of age-related changes in the human meniscus.[31-33] The relation between the presence of vessels, vascularity, and proper circulation of the meniscus should be assessed. Acknowledging the impact of the knee joint's surrounding environment on blood flow to the meniscus is equally crucial.

The results of this study indicated that the meniscus showed no active blood flow, even in the red-red zone. This finding supports the notion that blood flow in the red-red zone cannot be initiated without synovial stimulation. The meniscus was poorly vascularized, even in the red-red zone. However, blood perfusion inside the meniscus might be activated by synovial stimulation. The optimal ICG dose to observe vascularity during knee arthroscopy can be diluted by 10× in a 2.0-mL intravenous injection. However, the dose may be changed based on the physical status of each future clinical case. To accurately detect fluorescence, considering the correlation between the depth of the target tissues and the intensity of ICG fluorescence is crucial, as are the angle and distance at which a NIR light is directed onto the tissues. These factors bear great significance for future clinical applications.

Currently, ascertaining the significance of the fluorescence observed in the meniscus is challenging. However, this study might present a translational finding to elucidate the case-specific factors of meniscal vascularization in the near future. The results of this experimental study support the participation of synovial vascularity in the meniscus and corroborate the classic literature by King[17] and Arnoczky and Warren.[1,18]

The greatest advantage of ICG fluorescence-guided arthroscopy may be observing blood flow—which until now could only be confirmed in cadavers—in real time under arthroscopy. To fully comprehend the value of ICG fluorescence-guided knee arthroscopy, accumulating its clinical applications is necessary. As previously reported, in a study documenting clinical experience since 2018, fluorescence emission has been observed approximately 30 to 40 seconds after intravenous injection of 2.0 mL of 10× ICG solution, and fluorescence can be visualized within approximately 15 minutes.[34] The duration of fluorescence visualization is too short for evaluation, and the investigation should be conducted within the time frame of fluorescence. The application of ICG fluorescence-guided arthroscopy may facilitate the visualization of detailed blood flow in the knee joint and could be considered a future option in the decision-making process in cases of meniscal surgery.

Limitations

The primary limitation of this study is that only 1 pig was used to evaluate the medial compartment. The size of the pig's joint precluded assessment of the lateral compartment. Additionally, the potential for confounding due to the use of different ICG dilutions at different times in the same specimen cannot be entirely eliminated. Furthermore, the absence of a histologic examination of the fluorescent tissue vascularity of the meniscus in this pig model limits the findings of this study.

Conclusions

The optimal dilution and dose setting of ICG for knee arthroscopy was 10× in a 2.0-mL intravenous injection. The meniscus showed no active blood flow, even in the red-red zone. This finding might support the notion that blood flow cannot be initiated, without synovial stimulation, even in vascular areas.

Disclosures

The author reports no conflicts of interest in the authorship and publication of this article. Full ICMJE author disclosure forms are available for this article online, as supplementary material.

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