

## Casting Hind Legs of Rabbits after Full Transection of Achilles Tendons- Impact of Cast's Color and Composition

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### Abstract

Preclinical animal models as the rabbit Achilles tendon transection model demand for accurate post-operative treatment. Immobilization may be realized by application of a cast, the angle of which can be precisely managed and changed upon re-casting. Having a cast on the hind leg is per se not very comfortable and although the casts are very well padded, rabbits react towards casts by nibbling and biting into the cast material. Interestingly, the color and composition of the cast material seem to have an impact on how much New Zealand white rabbits nibble and bite into the material. It was found that especially dark blue casts were nibbled and bitten into until full destruction while red and violet casts were also severely bitten into, but less damaged than dark blue ones. Black casts were practically untouched and left as they were. While black casts did not have any fiberglass in their chemical composition, dark blue, red and violet casts had a fiberglass component. Hence, if casting the hind leg in the rabbit model is necessary, neither the color nor composition of the cast should be neglected from our findings as presented here, it should (i) not contain any fiberglass component and (ii) not be dark blue, but rather black in order to prevent the problem of biting it or nibbling during the phase of immobilization.

**Keywords:** Rabbit; Cast; Hind leg; Achilles tendon; Color; Black; Dark blue; Red; Violet

### Introduction

Preclinical animal models like the rabbit Achilles tendon full transection model are commonly used to study therapies or treatments that are designed to improve tendon surgery and rehabilitation [1-3]. Although early active mobilization helps the transected Achilles tendon to regenerate and heal [4], immobilization is still often used at least at early time points post-operation either by application of botox [5] or by a common cast [6].

Cast application involves many steps and hurdles like the appropriate tightness of the cast to avoid swelling of the limbs, the padding with soft tissue in order to prevent rubbing against the very sensitive and vulnerable rabbit skin (large clipped surface area for surgery) and also the optimum ankle angle required for an adhesion free tendon regeneration. For example, adhesion formation to the surrounding tissue during the healing process has been shown to be minimized by re-casting the Achilles tendon with a smaller angle after a 3-week period [6]. Nevertheless, if casts are optimized from a healing point of view, the tolerance by the animals may differ a lot and in a parameter that is not primarily important to the clinical setting, among others: the cast's color. Furthermore, we discuss implications of the cast's composition on its cushiness and being tolerated by rabbits.

## Materials and Methods

### Animals

For this in vivo study, 9 Specific Pathogen Free (SPF) female New Zealand White rabbits aged 12 to 16 weeks were used (Charles River, Research Models and Services, Germany). All animals were housed in social groups of four individuals in four interconnected cages, each of them with a bottom area of 70 cm x 70 cm and a height of 62 cm (Indulab, Switzerland). The animals were maintained under controlled conditions: temperature  $22 \pm 1^\circ\text{C}$ , 45 % relative humidity, 15 air changes per hour and a light/dark rhythm of 12 hours. The rabbits had free access to water (automatic water supply), autoclaved hay and straw ad libitum and to standard pellet diet (Kliba Nafag, Nr. 3410, Provimi Kliba AG, Switzerland). Ethical approval for the experiments was obtained from the veterinary office of Zurich, Switzerland (reference numbers 92/ 2009 and 193/ 2012 and 225/2015). Prior to surgery, all animals were acclimatized to their environment for 3 weeks. Four further naïve animals without surgery (not linked to that experiment) were given balls made of casting material (see below) as additional enrichment.

### Achilles Tendon Repair and Cast Application

The rabbits received premedication with 65 mg/kg body weight Ketamine and 4 mg/kg Xylazine. A venous catheter was inserted in the marginal ear vein. The rabbits were intubated with Propofol i.v. 1.0 mg/kg. Anaesthesia was maintained with 1-2% isoflurane. In order to ensure systemic analgesia during the time of operation, 0.2-0.3 mg/kg body weight Butorphanol (Dr. E. Graeb AG, Berne, Switzerland) was applied pre-operatively. The hind legs were shaved and cleaned with iodine. The Achilles tendon exposure was obtained through a paratendineal incision of cutis, subcutis and fascia. The medial and lateral M. gastrocnemius of the Achilles tendon complex were then sliced perpendicularly to the length of the tendon 2.0 cm above the calcaneus and the two tendon stumps were sutured (4-strand Becker suture) using a USP 4.0 polypropylene thread. Subsequently the wound was closed with a running suture (using a USP 6.0 polypropylene fibre) of the fascia and interrupted skin. Immediately post surgery, a Durogesic Matrix patch (Janssen-Cilag AG, Switzerland) was applied with 4.2 mg Fentanyl per patch in order to provide analgesia for about 72 hours with 25 µg/h Fentanyl.

Postoperative treatment included a first cast having an angle of  $180^\circ$  at the ankle. The angles at the ankle (from the knee to the ankle and from there to the toes) were measured with a goniometer. The cast was well padded: After application of Bactigras®, Elastomull® and cohesive bandage, a stocking was pulled over the leg. Then Terry Pad was used to pad it dorsally and ventrally. After a base coat with soft bandage, a Scotchcast™ conformable splint was applied dorsally as well as ventrally and covered by another softcast bandage (either dark blue, red or violet, all from 3M, (3M GmbH, Switzerland) or a black one from Nemoa, Allensbach, Switzerland). While soft-cast bandages from 3M had a fiberglass component, the one from Nemoa did not have any. The cast was changed after 3 weeks and the second cast removed after another 3 weeks. The second cast had a smaller angle of  $150^\circ$ . Great attention was

paid to ensure that the cast was not too tight so that it would be well tolerated by the rabbits (no swelling of the digits).

### Treatment Groups

The 9 rabbits were randomly distributed into 3 groups with  $n=3$ . All were operated on one hind leg [7]. The rabbits received either a dark blue ( $n=3$ ), a black ( $n=3$ ), a red/ violet cast ( $n=3$ ). Red and violet casts were mixed because in one case the violet casts were running out and a red had been used to close the defect. As the rabbits were re-casted after 3 weeks, the total number of casts amounted to a representative  $n=6$  of each color.

### Scoring of Cast Destruction

As some of the casts were almost completely destroyed while others were left untouched, we used a semi-quantitative scoring system for the extent of cast destruction: 0=no nibbling; 1=a little nibbling; 2=half of the cast nibbled into; 3=severe nibbling, more than half of the cast closed by tape (Tesa tape Switzerland AG, nr.4651, black, waterproof as well as adhesive and elasticated cotton "Pflasterbinde" Henry Schein, Switzerland).

### Balls Made of the Casting Material

In the two cages of four naïve rabbits left un-operated, four balls of differently colored casting materials were put on the cage floor; a dark blue, a red, a violet and a black ball (Figure 1). After eight days, the balls were taken out of the cage and the nibbling extent was inspected and scored semi-quantitatively (0=no nibbling; 1=a little nibbling; 2=half of the ball nibbled into; 3=severe nibbling, more than half of the surface nibbled into).

### Statistics

Data were analysed with Stat View 5.0.1 (SAS Software). One-way analysis of variance (one way ANOVA) was conducted to compare the different colour groups. Pair wise comparison probabilities (p) were calculated using the Fisher's PLSD. P values  $< 0.05$  (\*) were considered significant. For  $p < 0.01$  \*\* and for  $< 0.001$  \*\*\* was used. Values were expressed as means  $\pm$  standard deviations.

## Results

### Casts on the Hind Leg

While the black casts were almost untouched after three weeks, the red and violet casts had been nibbled into and some markedly damaged; however, the dark blue casts were bitten into so severely that the animal caretaker (daily observation) had to help out with tapes. No small pieces of the cast had been left in the cages and found by the caretaker we conclude that the rabbits not only bit into the casts; indeed they were also seen chewing and swallowing small pieces of them. No digestive trouble or changes in the fecal pellets was noted. After 3 weeks, the dark blue casts were mostly destroyed and only kept in shape by tape around the gauze padding and splint (Figures 2A and 2B). The dark blue cast was not only bitten into but severely destroyed. As for the six dark blue casts, all were bitten into and exhibited large defects, while the black casts were hardly touched or nibbled by the rabbits, although all casts were applied in the same way (M&M section) (Figures 2 C and 2D).

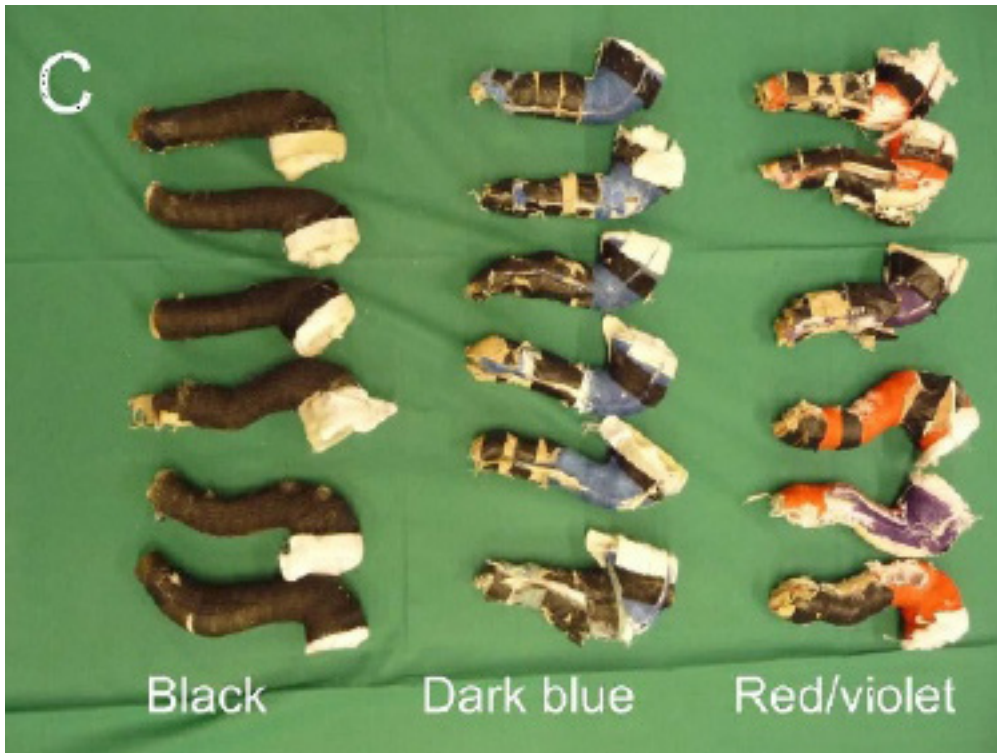


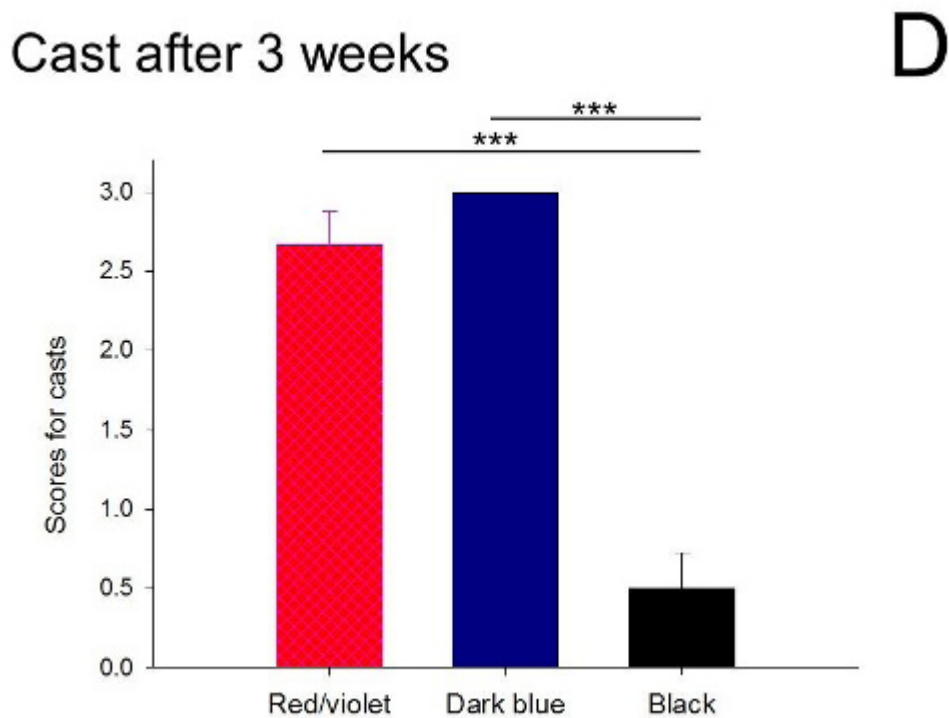
**Figure 1:** Balls made of the casting material that was used to cast the rabbits' hind legs. The balls were placed into two out of four cages shared by a social group of the four naïve rabbits as an enrichment. While soft cast bandages from 3M (red, violet, dark blue) had a fiberglass component, the black one from Nemoa did not have any.

# A



**B**





**Figure 2: Casts of the three groups.** A and B Rabbits in the cage wearing a black and a red cast or a blue one, respectively. C group on the left: black casts; in the middle: dark blue and on the right side: red/violet. D Semi-quantitative scoring for the destruction of the casts after 3 weeks. Obviously, black casts have been almost left intact by the rabbits, while blue, red and violet casts have been bitten into and nibbled. While soft cast bandages from 3M (red, violet, dark blue) had a fiberglass component, the black one from Nemoa did not have any. One-way ANOVA with  $p < 0.05$  (\*),  $p < 0.01$  (\*\*) and  $p < 0.001$  (\*\*\*).

### Reaction towards Casts as Balls

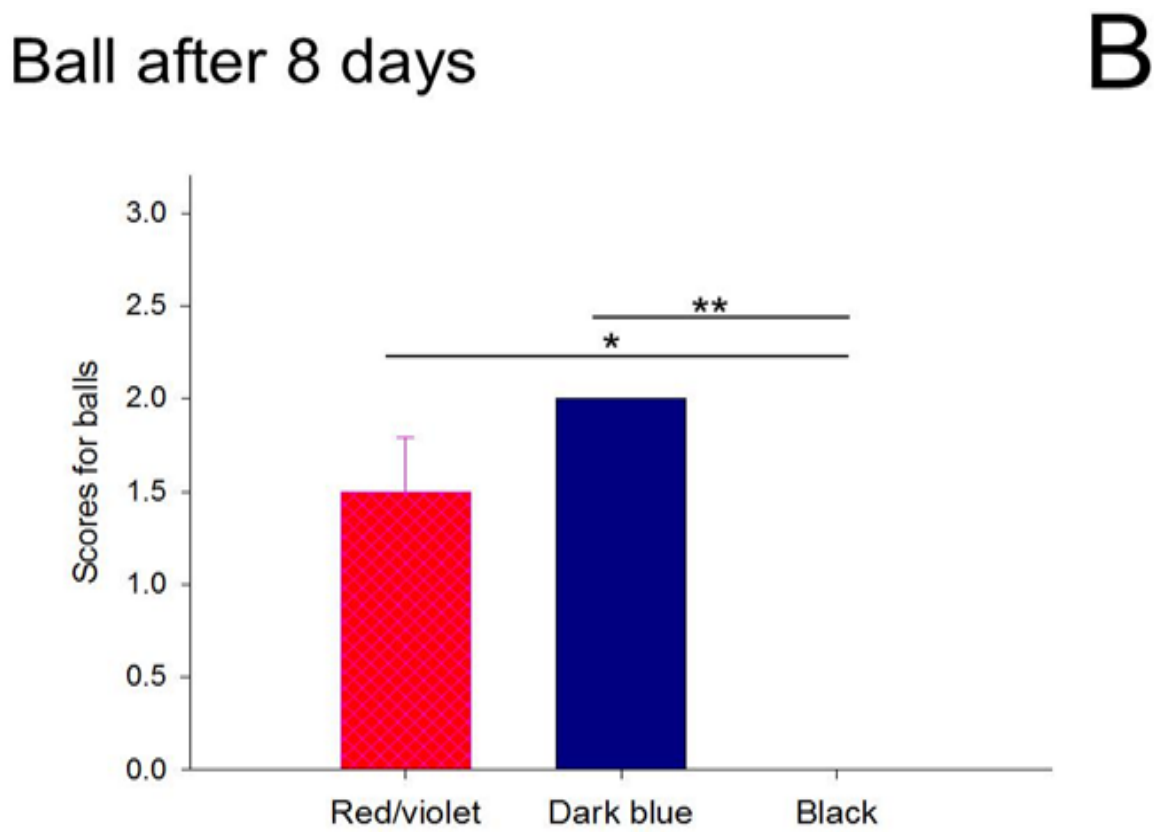
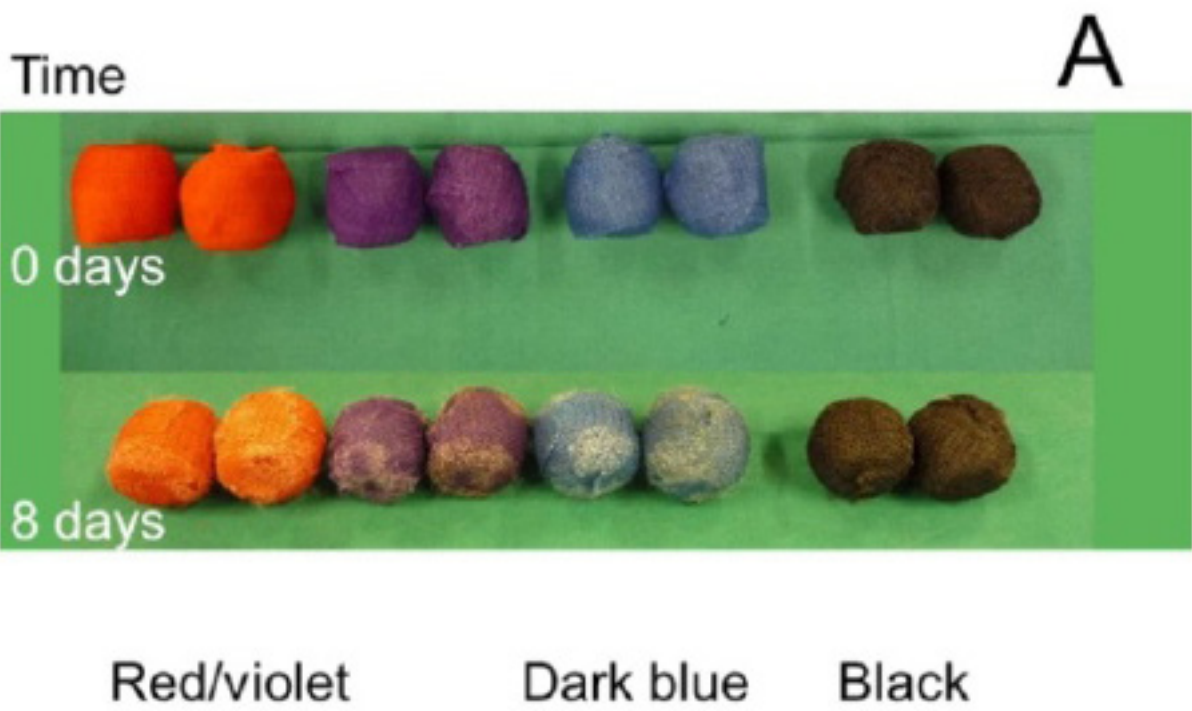
The four naïve rabbits reacted in a similar way towards balls made of casting material placed in their cages. The black balls were left completely untouched, whereas the red, dark blue and violet balls were nibbled into already after eight days (Figure 3).

### Discussion

Here, we describe for the first time that the color and composition of the cast used in animal experiments may have an impact on post-operative feasibility and treatment outcome. While female New Zealand white rabbits that had been operated on their Achilles tendon by full transection and well established, commonly used sutures, bit into and destroyed dark blue casts so that they had to be reinforced by the animal caretaker, they did not react adversely towards black casts. As for the red and violet casts, they were also bitten into, but not completely destroyed as the dark blue ones. We were very surprised about this observation first because we observed that only some of the rabbits nibbled severely on their casts. As it turned out to be preferably the same colors (dark blue, and to a lesser extent red and violet, but never black), we judged this observation worthwhile reporting to the community working in this field, because it is easy to choose another color than

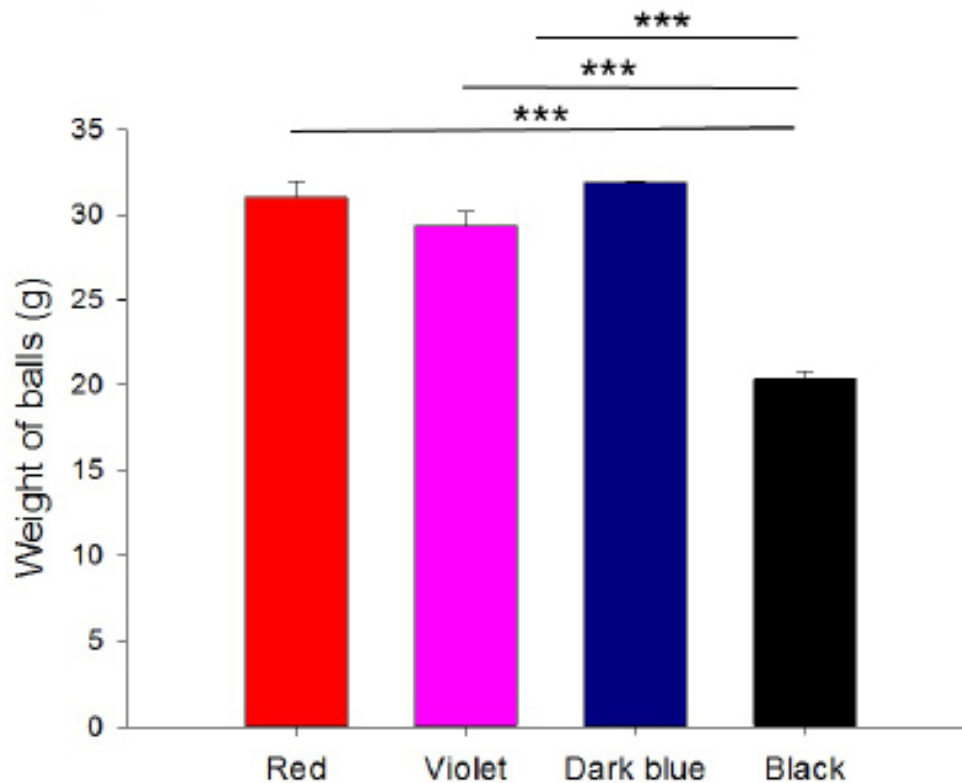
dark blue, red or violet avoiding trouble-shooting with a damaged cast, thus maintaining its initial cast function.

Furthermore, in order to support this observation about biting preferably into dark blue, red and violet casts, we also determined the reaction of un-operated rabbits towards balls made of the same cast material and the four chosen colors. Likewise, the dark blue balls were nibbled at more than the red and violet ones, while the black ones were left untouched. Rabbits have been reported to have fields of vision with different color perception based on the distribution of the cones in the retina [8]. Interestingly, rabbits have a blue streak of vision in the lower part of the retina, while if they look straight forward, more long-wave colors such as red-green are perceived. The high density of blue cones in the retina could possibly affect the way they perceive their casted leg, in particular during the grooming process of ano-genital region and toes. Furthermore, natural preferences of rabbits for white and yellow colors have been reported during growth, while green and especially blue are not preferred [9]. It might be speculated that it is the color blue itself (besides the inconvenient cast) that annoys and disturbs the rabbit so that it bites into it, which was supported by our second experiment where the cast material was exposed to the rabbits in form of a ball placed in their cages. Again, dark blue balls were attacked more frequently and severely than the other colors, with the black color being the least attacked.



## Weight of balls

# C



**Figure 3: Balls made of different casting materials.** A Balls freshly prepared and after 8 days distributed in two cages of two rabbits each, B Semi-quantitative scoring for the destruction of the balls after 8 days and C Weight of the freshly prepared balls. While soft cast bandages from 3M (red, violet, dark blue) had a fiberglass component, the black one from Nemoa did not have any. One-way ANOVA with  $p < 0.05$  (\*),  $p < 0.01$  (\*\*) and  $p < 0.001$  (\*\*\*)

Another explanation might be found in the composition of the cast. While the dark blue, the red and the violet casts exhibited a fiberglass component, the black one from the company Nemoa did not contain any fiberglass. This difference results in different stiffness and weight of the cast material; while dark blue, red and violet casts were stiffer and therefore a little bit less comfortable to be worn on the hind leg, the black cast was softer and probably led to a better shape adaptation around the leg. Moreover, fiberglass containing casts (dark blue, red and violet) were one third heavier by weight, which might add up to the annoyance perceived by wearing a cast on the hindleg.

Further compounds' analysis was withheld from us due to the companies' secret of material composition. Neither company, 3M (dark blue, red and violet casts) nor Nemoa (black casts), were prepared to reveal the exact chemical composition of their cast materials. Hence, although rabbits bite primarily into dark blue casts (full destruction) and to a lesser extent into red and violet ones, the complete rejection to bite into black material might be attributed more to the lack of fiberglass than to the color.

This reveals one limitation of this short communication having casts of the same color with and without a fiberglass component. But as we had just taken four different colors without intention to study the color, it was by chance that the black one did not have a fiberglass component (the 3M company does not sell black ones). Nevertheless, the differences were so striking that also other aspects were taken into account to explain the observation. Rabbits like to sharpen and whet their teeth on rough and stiff surfaces, which might also account for the fact that rabbits primarily bite into fiberglass composites. Thus, if the color and composition arguments are taken together, the dark blue cast including fiberglass was the worst for the rabbits (in terms of comfort) and therefore mostly attacked and bitten into, while the black fiberglass-free cast was worn best and left intact during three weeks.

## Conclusion

From our observations in this study, we suggest using black casts from Nemoa, and avoid dark blue ones of the 3M to cast the hind leg of rabbits.

## Conflict of Interest Statement

There are no conflicts of interest.

## Acknowledgements Statement

We thank the people from the “cast room” at University Hospital Zurich for helping out with casting the rabbits.

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