The transdermal penetration of novel amphiphilic MTC-Y carrier

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Research Article

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Posted Date: April 28th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-440487/v1

The transdermal penetration of novel amphiphilic MTC-Y carrier.

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Abstract

A transdermal drug delivery system capable of transporting the active substance through skin is alternative path for drug delivery for different purposes. In an attempt to demonstrate the permeability of the active substance through the skin layers of the rats, the amphiphilic MTC-Y carrier was combined with fluorochromes of different chemical properties. After extraction, the skin material was subjected histological examination under fluorescence microscope Nikon Eclipse 80i UV-2A filter (EX330-380, DM-400, BA-420). Moreover, histological slides routinely stained with haematoxylin were analysed. Results indicate that use of the MTC-Y carrier seems to be very promising compound for drug delivery both locally and systematically

Introduction

A transdermal drug delivery system capable of transporting the active substance through skin into the body is a very promising pathway for future. Topical administration of drugs offers manyadvantages over conventional oral and invasive techniques of drug delivery. Importantadvantages of transdermal drug delivery are prevention from metabolism in gastrointestinal system including liver,enhancement of therapeutic efficiency and maintenance of steady level of the drug in the blood. On the other hand, the administered drugs may act locally (muscles, dermis), avoiding blood circulation and reduce the its accumulation in the organs or tissues[1,2].

The skin barrier function has been attributed to the stratum corneum and represents a major challenge in clinical practice pertaining to cutaneous administration of drugs. Despite this, a large number of bioactive compounds have been successfully administered via cutaneous administration because of advances in the design of topical and transdermal formulation[3,4].

In the development of transdermal and topical products it is important to understand how formulation ingredients interact with the molecular components of the upper layer of the skin, the stratum corneum (SC), and thereby influence its macroscopic barrier properties.

The skin is composed of 3 layers- epidermis (ED), dermis (D) and subdermis or subcutis (SD) a layer responsible for contact between dermis and the rest of the body. This layer is mainly composed of loose connective tissue with different amount of adipocytes. In this layer, topically administered drugs if are hydrophobic may stuck in the adipocytes. In this layer, topically administered drugs may stuck in the adipocytes. The dermis, composed of dense irregular connective tissue contains numerous collagen fibers running in different direction among which blood vessel and collecting ducts of skin sweat gland can be found. Additionally, hair roots with sebaceal glands can be visible in different concentration depending on localization. In this layer, drugs are believed to enter blood circulation, or depending on demands act directly on cells (inflammatory cells, cancer cells etc.)[5].

The epidermis consist of two layers: stratum corneum (SC) and viable epidermisor germinative layer (GL) where cells divide and differentiate into the keratinized cells of SC[6].The epidermal barrier protects the body from excessive water loss and the entry of exogenous substances. An essential component of the permeability barrier is the extracellular lipid matrix of the SC.This lipid matrix consists of numerous lipids including ceramides, free fatty acidsand cholesterol. Any changes in lipid content may vary the permeability of the SC [7,8].

To penetrate skin barrier, carrier compound should locally obliterate all mechanical, physical or chemical obstacles. Numerous skin penetration enhancers are proposed to improve topically applied drug delivery. Hybrid terpene-amino acid enhancers, esters of terpene alcohols, niosomesdendrimer nanoparticles, peptides[8–14].The another method for increased/decreased skin permeability is the use of mechanical factors such as electric devices or ultrasound, iontophoresis, sonophoresis[6,15,16].

The effectiveness of the formulation applied on the skin depends not only on the biologically active substances used in it, but also the permeability of the ingredients through the skin. Even in the case of a high-class, modern formulation, to ensure it works it must penetrate SC, which is most demanding barrier in most species[17].

Penetration through the epidermis is possible by two ways: the transepidermal route, penetrating through either through the epidermis or through the dermal appendages, i.e. the sweat glands; and the sebaceous glands and hair follicle[3,18].

Magainin, a naturally occurring pore-forming peptide, was found to increase skin permeability by direct interaction with and disruption of stratum corneum lipids. Among the possible approaches to screen new skin penetrating peptides, the use of phage display peptide libraries is gaining a growing

interest since they allow identifying a specific peptide able to penetrate the skin and to carry filamentous bacteriophages through the stratum corneum[19].

Therefore the aim of this study was demonstration of the permeability by the skin chosen active compounds[8].

Material and methods

In an attempt to demonstrate the permeability of the active substance through the skin layers and into the cell interior, the active substance was combined with fluorochromes of different chemical properties, characterized by different ability to penetrate into the interior of cells present in the skin and subcutis.

The experimental carrier **MTC-Y** designed by BIOTTS S.A. was used to transfer active substances through skin layers without affecting their homogeneity.

- MTC-Y 1O – Patent application No. **P.428534** pH 3,5-5,0 carrier: non-aqueous emulsion containing mixture of amphiphilic imbibition promotors, containing as lipophilic phase oleum chaulmoogra.

- MTC-Y 1Z – Patent application No. **P. 430531** pH 9,8-10,5, non-aqueous emulsion containing mixture of amphiphilic imbibition promotors, containing as lipophilic phase Adeps suillus and oleic acid**.**

- MTC-Y 1LCO – in the process of preparing a patent application pH 3,5-5,0 carrier non aqueous emulsion containing mixture of amphiphilic imbibition promotors, containing as lipophilic phase Adeps suillus and oleum chaulmograwith addition of lecithin and cholesterol.

The study was approved by a local Ethics Committee for Animal Experimentation (No. 021/2019/P1) in Wroclaw. All experiments were performed in accordance with relevant guidelines and regulations and follows the recommendations in the ARRIVE guidelines.

Preliminary results of 1% fluorescein incorporated in a modified MTC-Y carrier to obtain systemic action through the dorsal area of experimental animals in 2-day observation. The material was divided into 3 experimental groups of 3 animals each. The animals were kept individually in cages equipped with a vivarium of the Department of Biostructure and Animal Physiology, Wroclaw University of Life Sciences.

After shaving the skin, the semisolid product was applied on the dorsal part of the body and was kept for 2 days. After this period, the animals were sacrificed and the samples of skin, adjacent muscles, liver and kidneys were taken for examination.

Macroscopic examination of sawdust present in the cage using a UV flashlight (395nm) showed the presence of a fluorescent substance in urine about 30 minutes after application.

After extraction, the skin material was subjected to equilibration at 4°C for 1 hour and then frozen in the fridge at temperature of -40°C. The material was cut in a cryostat at -30°C from the inside to the outside in order to avoid the transfer of particles marked by the blade of the knife. The 10 μm thick slides were fixed in 70% alcohol and enclosed in Euperal^{tm}. The material was analysed with a Nikon Eclipse 80i fluorescence microscope using a UV-2A filter (EX330-380, DM-400, BA-420). Moreover, histological preparations routinely stained with haematoxylin and eosin were made from these fragments.

The study of the nine days observations was performed on Buffalo rats, randomized and kept separately in cages with toys in standard conditions in the vivarium of the Department of Biostructure and Animal Physiology. The animals were divided into 12 groups of 3 animals each.

The same procedure was used with the use of 1% Fluorescein ($C_{20}H_{12}O_5$), 5% Acridine orange ($C_{17}H_{19}N_3$) and 5% Rhodamine B ($C_{28}H_{31}N_2O_3Cl$) (Sigma Aldrich) mixed with the carriers

The skin and adjacent muscles, liver and the entire hip joint were taken for examination.

Results

Histology

Routine microscopic analysis did not reveal any significant direct effect of the substance on tissues. Both in the control and experimental samples, no changes in the vascular bed, leukocytic infiltrations or necrotic areas were found in the skin. The epithelium covering the skin was normal and consisted of several layers of cells of weakly keratinized multilayer flat epithelium. The reproductive layer did not show any intensive proliferative processes. In the subcutaneous and adjacent tissues, despite the presence of the active substance (fluorescein), no pathological changes were found, like in the kidneys and liver.

In 9 day observations haematoxylin and eosin staining did not revealed hypertrophic, necrotic or immunological changes in the place of application of the compound in a semi-solid form containing the MTC-Y carrier and the active substance (selected fluorochromes).

The 2-day permeability analysis of the modified MTC-Y carrier with fluorochrome (fluorescein) indicates rapid penetration of the molecule through skin layers into the liver and kidneys. The first observations of fluorescein in urine were observed as early as 30 minutes after the beginning of the

experiment. Fluorescein was organically accumulated in the dermis, much less in the muscles and subcutaneous tissue. The images of skin and muscles were modified in a graphical program to visualize the structure of the skin. Kidney and liver photos were not modified graphically. It is worth noting that this observation confirmed the rapid penetration of the active substance through the skin, and its limited accumulation in the dermis and liver, where it was metabolized for at least a few days.

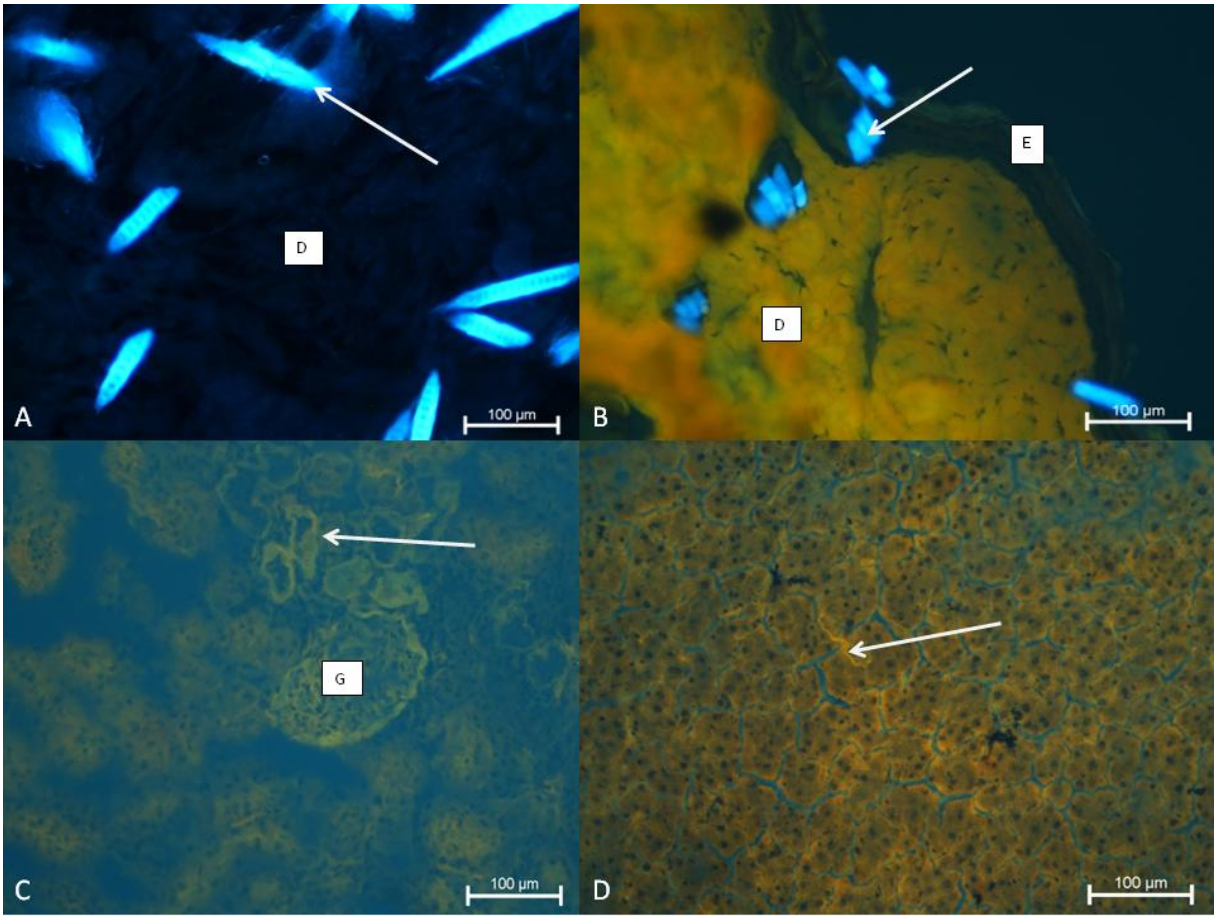

Fig 1. Skin changes in two day observation. A- control skin (without fluorochrome) Visible natural hair fluorescence (arrow). The background collagen fibres of the dermis (D) are practically invisible. Mag 200x.; B- MTC-Y 1Z carrier combined with fluorescein. Positive reaction in skin (D) and weak in epidermis (E). The blue fluorescence of hair is preserved. Photo after modification of colour intensity in Nis elements Ar. Mag 200x; C- Kidney, D- Liver. Positive reaction in liver and kidney (arrow). A photo without modifying the colour intensity in the Nis elements AR software. Mag 200x

The other two fluorochromes enclosed in the MTC-Y medium show slightly higher affinity to cells. Acridine orange is slightly bonded to muscle cells but is detectable e.g. in the liver, while rhodamine is easily bonded to cellular elements in the skin, including epithelial products (such as hair or skin gland

cells) and fibroblasts, while in this experimental system it poorly bonded with collagen and small cells present in the skin, such as fibroblasts, mast cells, macrophages or muscle cells. A study using acridine orange showed bone penetration with MTC-Y 1Z and MTC-Y 1O modifications. In the study the functionality of specific modifications was confirmed in the fluorescein study (See Fig 1).

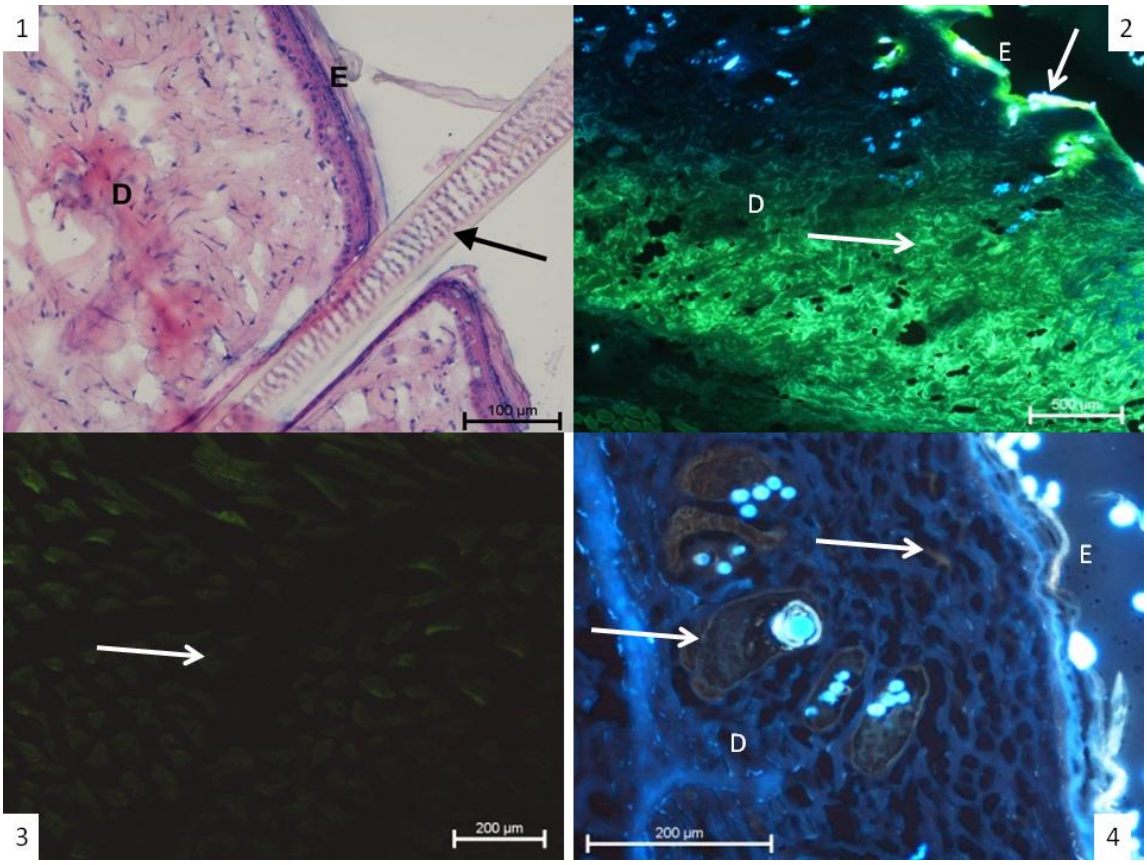

Fig 2. Skin samples from rats in 9- day observation. 1- normal skin fragment with hair (arrow) H&E staining, Mag 400x, 2- Fluorescein- visible positive reaction on skin surface and in dermis (white arrow) Mag 100x, 3- acridine orange, positive reaction in skeletal muscles Mag 200x, 4- Rhodamine B- positive reaction in sebaceous glands and in the cells if dermis. Mag 400x. D-dermis, E-epidermis.

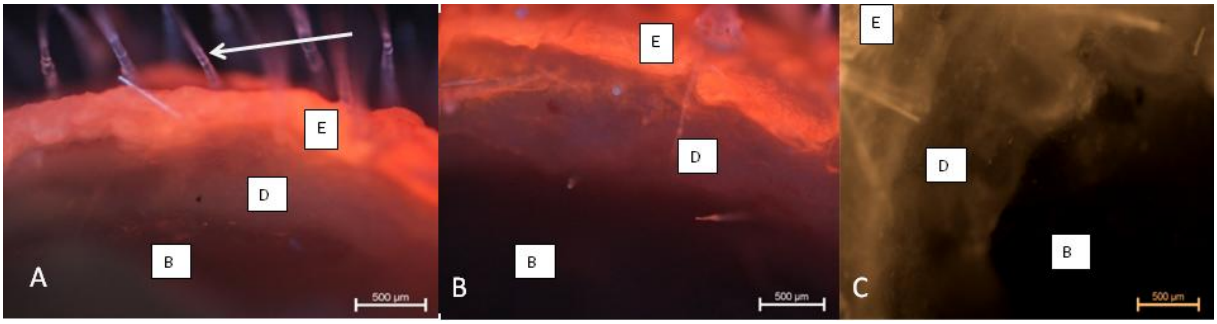

Fig 3. Example pictures from tail end. MTC-Y carrier combined with rhodamine in all three modifications showed a signal in the subcutaneous and adjacent bone tissues in groups 1.0 and 1Z. Visible blue fluorescence of hair (white arrow). Negative reaction in LCO group (photo modified graphically in the Nis elements AR software). Mag 200x

Hip joint tests did not show the presence of the fluorochrome in any of the tested cases. Therefore the results were not included.

Discussion:

Topical skin delivery of drugs is currently being employed for different purposes such as skin cancers treatment, inflammatory diseases, alopecia and topical anaesthesia.

Many drug products applied to the skin surface may penetrate to some extent into the skin layers, where their effects are expected[13,20]. This is the case for topical formulations for treatment of skin disorders such as acne and cutaneous inflammatory diseases that include dermatitis, erythematous lupus, and psoriasis. On the other hand, transdermal formulations release drugs that permeate through the skin and enter the systemic circulation. Transdermal therapy must ensure significant concentrations of the drug absorbed from the surface to reach effective plasma level. Permeation of drugs is targeted in some cases to body regions close to the action site, where a regional effect is expected, e.g., in the muscles, blood vessels, and articulations. In this way, the term “cutaneous absorption” is properly used to characterize the sum of the amounts of drug that penetrate and permeate the skin[5,15,20,21].

Because one of the most important functions of the skin is the separation of the interior of the body from the environment, there are a number of mechanisms that can limit the absorption of substances by the skin[20]. One of them is the existence of alternating hydrophobic and hydrophilic areas occurring in particular skin structures. If any substance gets through these layers, it can easily spread through the body together with the lymph and blood serum. The rate of removal depends on local conditions, including the concentration of the substance or, for example, the rate of blood flow[5,15].

The study showed that fluorescein, characterized by the highest permeability to tissues and cells, seems to be the best fluorochrome to visualize the permeability of an active substance incorporated into the MTC-Y carrier[22]. Its presence can be easily detected even in urine and consequently it is easy to show its presence in internal organs and blood. The study confirmed the influence of the MTC-Y carrier modification on the depth of penetration of the active substance enclosed in the carrier and the possibility of controlling the absorption into the general circulation. A surface (external) effect was observed in the case of MTC-Y 1 LCO modification and no absorption into the deep skin layers or general circulation was observed. In the case of MTC-Y 1 O modification, absorption into deeper

layers of skin and soft tissues was observed. No absorption to the general circulation was observed (no fluorescence in liver cells). Modification of MTC-Y 1Z showed the highest degree of absorption and penetration depth. The presence of fluorochromes in liver cells and in urine was confirmed after about 30 minutes. The analysis of the structure and physicochemical properties of individual fluorochromes additionally confirms the possibility of transfer of various active substances, e.g. of different molecular masses (particle sizes of fluorochromes studied from 265.36 to 479.01 D) and different character (lipophilic and hydrophilic), through the skin layers. The use of fluorochromes with different physicochemical properties and the possibility of modification depending on the needs and active substances used confirm the universality of the MTC-Y carrier.

Different drugs have been studied but usually passive skin delivery is not suitable due to drug molecular size and physicochemical properties that hamper their delivery to deep skin layers[4,6,7,20,21,23]. Currently, physical methods are available both in lab bench and in the clinic and thus different devices have been developed and applied successfully. For topical delivery, although many studies were already performed, systemic exposure is a concern. Formulation strategies, such as the use of delivery systems can potentially increase the amount of drug retained in viable epidermis and avoid systemic side effects.

From this point of view to use the MTC-Y carrier seems to be very promising carrier for drug delivery both locally and systematically.

Acknowledgements

The authors want to express their gratitude to BIOTTS S.A. for the samples required to conduct the tests.

Statement of authors:

We certified that presented result was not published in the other journal and all had contribution in presented paper

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Figures

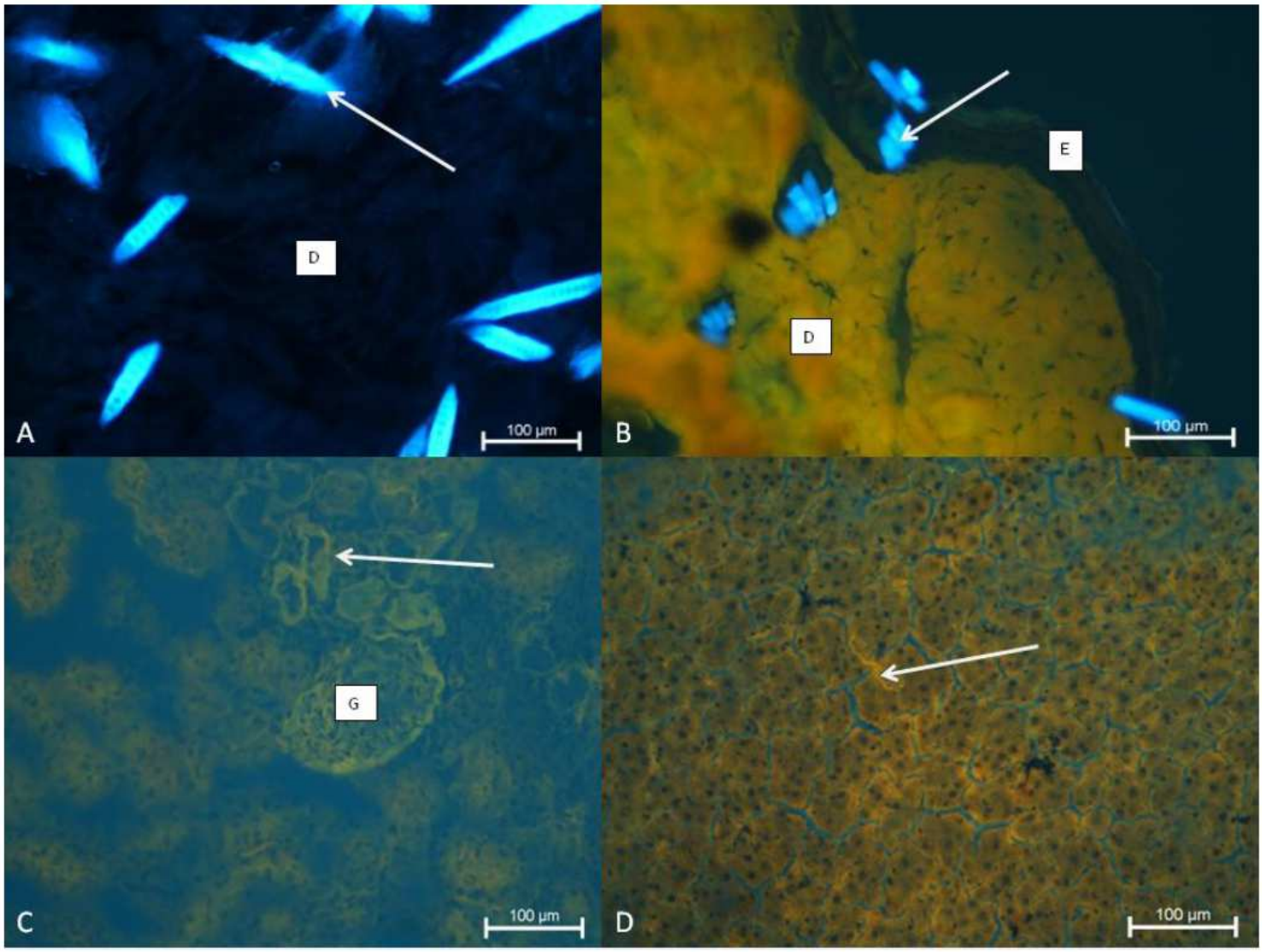

Figure 1

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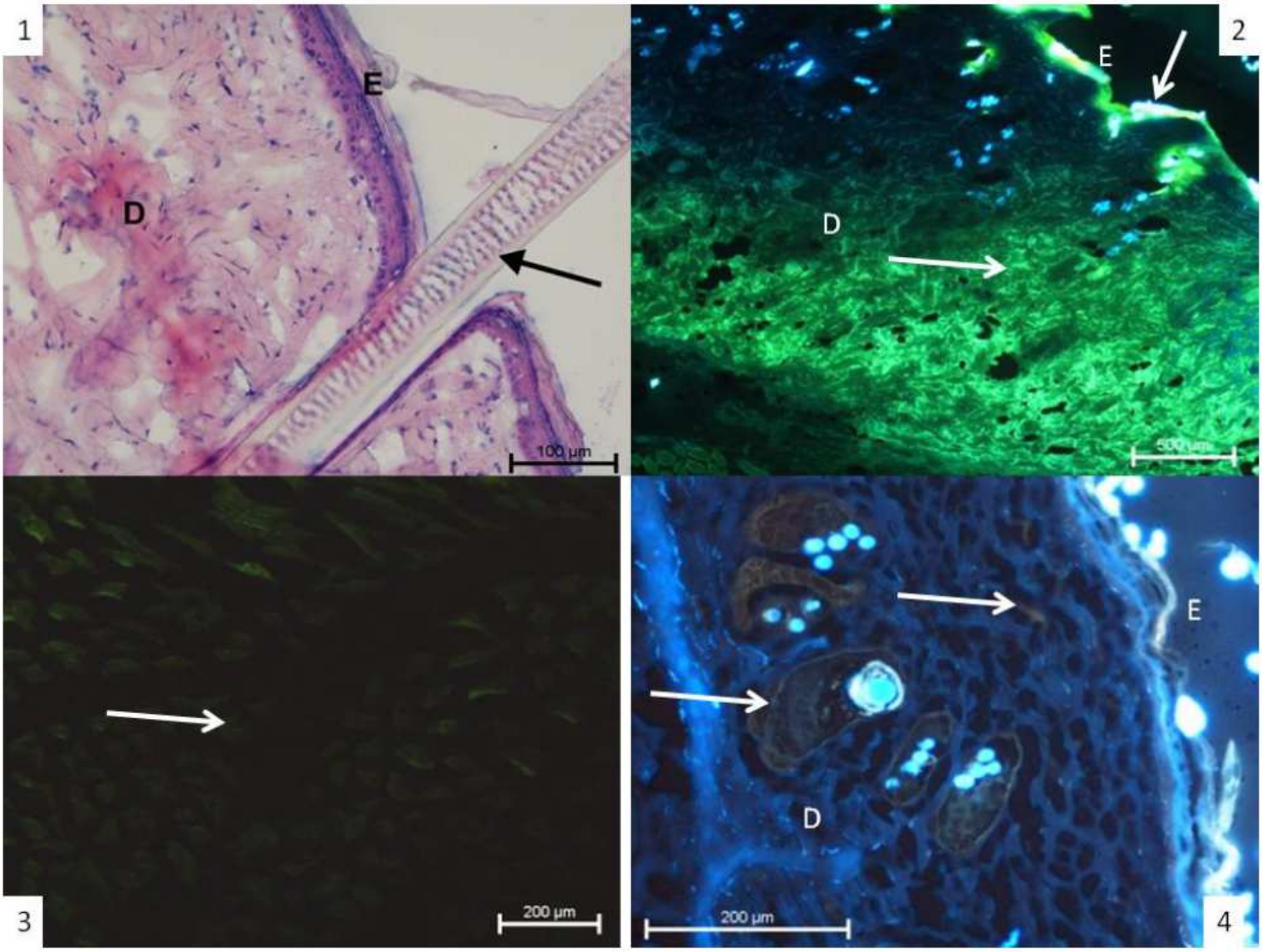

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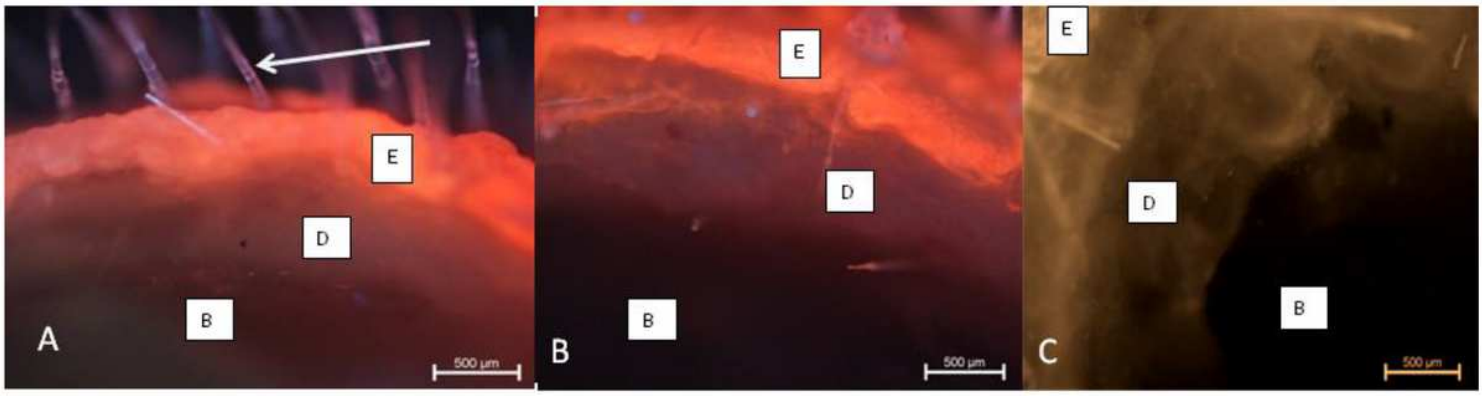

Figure 3

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