# SPECIAL REVIEW

# CONTROL OF MICROORGANISMS ON TANNED LEATHER: FROM FUNGICIDE TO ANTIMICROBIAL FUNCTION LEATHER

*by*

HAIBIN GU,[A,B]* LI ZHAO,[A,B] JUN MA,[A,B] YINGJIE YANG,[A,B] CHANGQING ZHAO[C] AND WUYONG CHEN [A,B]
[A]*Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University,*
CHENGDU 610065, CHINA
[B]*National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University,*
CHENGDU 610065, CHINA.
[C]*College of Bioengineering, Sichuan University of Science and Engineering,*
ZIGONG 643000, CHINA

## ABSTRACT

To inhibit the microbial growth on tanned leathers including wet-blue, crust leathers, finished leathers and their goods (shoes, garments, bags, etc.), fungicides are usually applied during various leather-making processes. Under the situation of increasingly strict environmental legislation, all kinds of eco-friendly fungicides were explored recently to replace the currently used noxious ones in leather industry. Meanwhile, a non-traditional new type of leather, called antimicrobial function leather, attracted more and more attention from leather chemists and technologists, because of its tempting antimicrobial protection provided and wide application prospects in many fields such as medical materials, health products, daily products, public transport vehicles, and so on. Based on the literatures published in the past decade, especially in the past five years, this review systematically and comprehensively summarizes current status and development trend about leather fungicides and antimicrobial function leather. The discussed antimicrobials contain traditional organic synthetic fungicides with small molecular weight, natural essential oils, macromolecular polymers and chitosan derivatives, and nano-inorganic antimicrobials (nanosilver, nano-ZnO, nano-$TiO_2$, nano-$SiO_2$, etc.). Finally, several proposals are addressed for the development of new leather fungicides and antimicrobial function leather, and especially, a new viewpoint, in which the antimicrobial leather is prepared based on various tanning mechanisms, is demonstrably presented to solve the problem of loose combination between antimicrobial substances and leather fibers.

## 1. Introduction

Microbial growth on leather, which is a processed product of natural animal hides and skins, is a constantly troublesome problem in leather industry. Normally, components in leather such as proteins, fats, oils, carbohydrates and minerals can serve as nutrients for the growth of microorganisms, especially bacteria and fungi. The former are mainly responsible for the decomposition of untanned proteins in raw hides and during soaking, while the latter frequently thrive on tanned leathers and their goods.[1,2] Microbial growth is highly negative not only to the quality of leather, but also to the health of tanners and consumers for leather goods. As shown in Figure 1, there is sharply loss in physical strength properties and irreversible aesthetic changes of leathers, which is mainly caused by the large reduction of fatty materials due to the degradation of fungi growing on leathers.[3] What is more, the damages to the contaminated leathers also contain decreased protein content, chrome content and hydrothermal stability, color marks, non-uniformity in further processing, and so on.[3,4] So, as shown in Figure 2, microbial control is indispensable during the manufacture, storage, shipping and using of leathers, and often carried out in many leather-making processes such as the storing of raw hides and skins, soaking, pickling, tanning, dyeing, fat-liquoring and finishing. This review will only focus on the microbial control on tanned leather, and the preservation of raw hides and skins is not included.

In the past decade, noteworthy changes appeared in the research field of control of microorganisms on tanned leather, which has attracted the attention of many leather chemists and technologists. (1) Increasing stringently environmental legislation obliged tanners to give up the use of some fungicides due to their toxicity or other issues, while others perhaps will be restricted in the future. Consequently, tanners are faced with a more limited number of fungicide products to choose from when it comes to these chemicals.[5] (2) Besides

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*Corresponding author e-mail: guhaibinkong@126.com; Fax; 86-028-85405237

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the conventional mold-proof of wet-blue was continually concerned, much attention was also paid on the microbial control of leather goods such as leather shoes, garments and furniture. (3) The purpose of microbial control is not only to protect the quality of leather and its goods whose deterioration will result in significant direct and indirect cost loss, but also to provide antimicrobial protection for consumers of leather goods. In other words, the corresponding leather is a function material, namely, antimicrobial function leather or antimicrobial leather that is more and more welcomed and focused in leather industry. (4) Fungicides and antimicrobials studied were not confined to the traditional synthetic small molecules, and as shown in Figure 3, all kinds of substances with antimicrobial activities such as natural essential oils, macromolecular chitosan, polymers and inorganic nano-materials were explored to be used as leather fungicides or to prepare antimicrobial leathers. According to the above changes, we will detail and systematically provide the state-of-the-art in the filed of microbial control on tanned leather and antimicrobial leather based on documents published in mainly past five years, and it is expected that this review is able to attract great interest of research and development in this area.

## 2. Synthetic Organic Micromolecular Antimicrobials

Antimicrobials with low molecular weight, which are prepared by the way of organic synthesis, are traditional leather fungicides frequently used to protect semi-processed (wet-blue, wet-white, vegetable tanned, or crust) and finished leathers against mold growth during storage and shipping. A good example is the 2-(thiocyanomethylthio)benzothiazole (TCMTB), as shown in Figure 4, that became the new standard of leather fungicide starting in 1970's and remained in use today.[2,5] It should be pointed out that most of fungicides often used in leather industry are highly effective to inhibit the troublesome fungal species growing on leathers although there were few reports about the microbial resistance caused normally by their improper application.[6,7] However, the main disadvantage of these fungicides is their toxicity to human beings and environment, and some of them were strictly limited or forbidden to be used in leather industry by the increasingly stringent environmental and health legislation. For example, dimethyl fumarate (DMF), which is an effective inhibitor of mold growth and often used in leather industry in the past, is a potent sensitizer that can cause severe allergic contact dermatitis (ACD).[8] According to the EU (2009/251/

Figure 1. Air-dried wet-blue showing grain rupture on the test (molded leather) and intact control.[3]

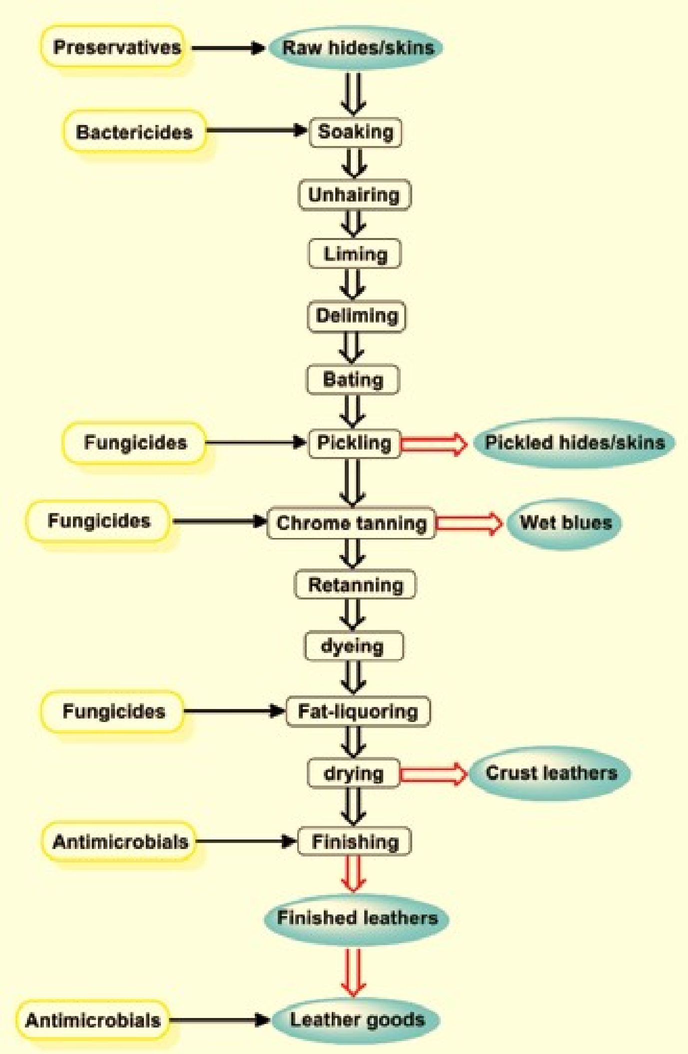

Figure 2. The normal technological process and microbial inhibition of leather manufacture.

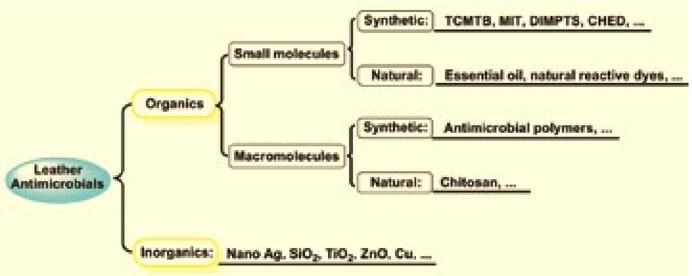

Figure 3. Types of antimicrobials studied in leather industry.

EC), the presence of DMF in products should be below the maximum limit of 0.1mg/kg.[9] Furthermore, TCMTB in tannery effluent was founded to be toxic to a population of *Mytilus edulis*,[10] and its toxicity to environment and human beings were reported.[11,12] More and more purchasers put forward the requirement about the maximum limit of TCMTB in leathers.

Under these circumstances, tanners were obliged to adapt their processes to alternative technologies with lower environmental impact. Normally, two ways were included in the development of new fungicide systems that comply with those environmental rules. One is the synthesis of new antimicrobial compounds with potential to be used as leather fungicides, or introduction of low-toxic and high-efficient fungicides from other industries such as food, cosmetic and textile. Another approach is the development of combined fungicides with two or more active ingredients that show synergistic effect on the fungal inhibition. For example, S-hexyl-S'-chloromethyl-cyanodithiocarbimate (CHED) was reported by Buckman[13] to be used as a new antifungal agent for the leather industry. CHED exhibits a very low MIC (minimum inhibitory concentration)(0.2 ppm against *Aspergillus niger*). As shown in Table I, a 30% CHED formulation were compared in laboratory tests with Busan 30L (30% TCMTB) as industry standard. At one-third lower offer, the CHED formulation gave similar performance results on full thickness wet-blue with no mold growth after 8 weeks exposure in the Tropical Chamber (TC). Furthermore, in production scale trials, a synergistic combination of CHED (Plus OIT and TCMTB) with total active substance concentration of 12.5% was run against Busan 30L (30% TCMTB) as industry standard. The results indicated that at approximately 30% lower offer, the CHED combination product (BLX-1608) gave also similar performance results on full thickness wet-blue with no mold growth after 8 weeks exposure in the Tropical Chamber. More importantly, CHED will degrade by hydrolysis and its byproducts show very low toxicity, and regulatory review has shown CHED to be as acceptable and in some cases it has proven more favorable than current industry active substances that are registered for use in leather manufacture in highly regulated regions like North America and Europe.[13]

Similarly, Zenith Chemicals[5] in Singapore developed a new azole-based antifungal agent, named as Azole 388*, specially for the leather industry, focusing on suitable existing active ingredients that could be used to develop new leather fungicides utilizing latest formulation technologies. Azole 388* was extensively tested in industrially produced leathers for fungicidal efficacy. The results of these tests over a period of nearly 5 years showed that this product consistently performed well, providing long-term mold resistance to the treated leathers.[5] As a viable new alternative for fungal control in leather making, this fungicide is formulated using a non-toxic, eco-neutral liquid carrier, which renders a product that is more eco-friendly than traditional solvent-based fungicide products that generate volatile organic compounds.[5]

Diiodomethyl-*p*-tolylsulfone (DIMPTS) and 3-iodo-2-propynyl-N-butylcarbamate (IPBC), whose structures are shown in Figure 4, were also developed as alternative fungicides for the leather industry.[14,15] They were applied in the chrome tanning process, fatliquoring of hides tanned with vegetable extracts and a preservative pickling process, and showed greater antifungal capacity than the conventional TCMTB and phenolic fungicides.[15] More importantly, the research work confirmed the highest level of toxicity of wastewaters of the wet-blue tanning process when using TCMTB, as shown in Figure 5, while the lowest toxicity of wastewater corresponded to the treatment with IPBC, which, in turn, provide the highest antifungal protection.[15] In addition,

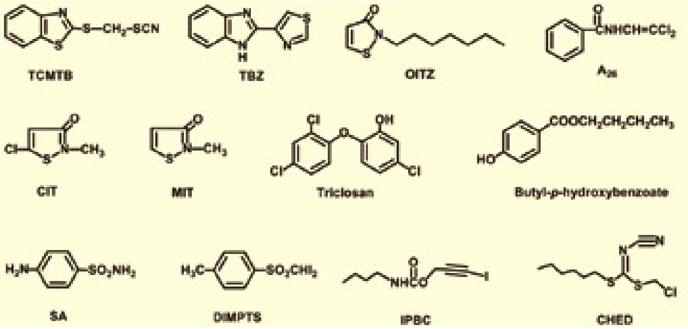

Figure 4. Some fungicides used in leather industry.

### TABLE I

### Results of laboratory trials on full thickness wet-blue-CHED (30% formulation) versus Busan 30L(TCMTB).[13]

| Product offer | Active substance[#] | TC[*]: 4 wks/8wks |
|---|---|---|
| 0.1% Busan 30L | 61 ppm | 10/10 |
| 0.07% CHED | 51ppm | 10/10 |

[#] Standard Buckman method: Solvent extraction of active substance followed by HPLC separation and quantification us UV detection against known standards. Values are corrected to 5 mm wet-blue thickness and 60% average moisture content.

[*] Standard Buckman method. Tropical chamber challenge test using optimum growth conditions and inoculation with known and wild strains of fungi. Leather sample coupons hung in the chamber are read weekly. A value of 10 indicates no fungal growth.

much attention was paid to the low-toxic isothiazolinone (Kathon - commercial name) with broad antimicrobial spectrum that is widely used in many industries such as cosmetics, painting, water treatment and paper-making.[16,17]

Recently, the fungal control of finished leathers and their goods attracted the attention of many leather researchers, especially tanners in China that is now the world's biggest exporter of leather goods. During transportation and storage, the problem of macroscopic mildew on leather goods such as shoes, garments, boxes, bags and gloves can cause huge economic losses to their manufacturers and distributors. Normally, antifungal processes include adding fungicides into finishing agents or shoe polish, putting special bags containing volatile fungicides into packing boxes, using antifungal packing materials such as mold-proof papers and bags, and employing commercial mildewproof sheets.[18] The strict restriction on the use of DMF, which was the most common used fungicide in the past, forced tanners to develop new alternative products with low health impact. In this field, the combined fungicides, which usually exhibit higher antifungal activities and lower toxicity than products with single active ingredient, were preferred and extensively studied. For instance, Gu et al. [9] developed a new combined antimicrobial agent containing triclosan, butyl-*p*-hydroxybenzonate (BHB) and the zinc complex of 2-amino benzothiazole and *p*-formyl phenoxyacetic acid ($Zn(SD)_2$), and applied it to prepare antimicrobial papers used to protect leather goods. Notably, the median lethal dose ($LD_{50}$) of $Zn(SD)_2$ is more than 5000 mg/kg according to the acute skin toxicity experiments on rats, and other two components are also low-toxic antimicrobials. So, we have the reason to believe this product will have wide application prospects. Meanwhile, Gu *et al.*[18] also prepared mildewproof sheets for leather goods using a commercial fungicide SCG-II developed which contains natural antimicrobial substances with slow volatile properties. As shown in Figure 6, in a harsh volatilization mildewproof experiment, the prepared mildewproof sheets revealed better mold-proof activities than the commercial products MICROGARDE and WASAOURO. Furthermore, many new eco-friendly combined antifungal agents, such as the mixing of imidazole, salicylic acid, sulfanilamide and benzalkonium bromide,[20] and the patented combined formula of IPBC and gallnut extract,[21] could inhibit the mold growth on leather goods.

In the past few years, organic antimicrobials found wide application in the preparation of antimicrobial leather that is a function material increasingly focused on. In general, there are two types of function leathers with antimicrobial performance. The first one is surface-antimicrobial leather that is normally prepared by coating the finishing agents containing antimicrobials. The leather obtained could be used to produce, for example, seats with antimicrobial hand-touch surfaces for application in the public place such as hotel, hospital, buses, trains, trams, stations, and so on.[22] The second one is the leathers with antimicrobial agents distributed in the whole cross-section, not just on their surface. A good example is the shoe lining leather exhibiting antimicrobial property, which can be used to inhibit the growth of pathogens in leather shoes.[23]

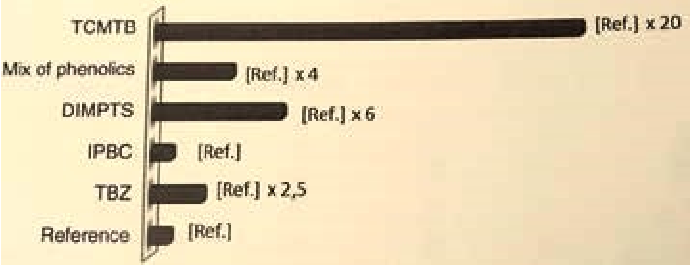

Figure 5. Toxicity of wastewaters of the tanning processes and fungicides with respect to the reference.[15]

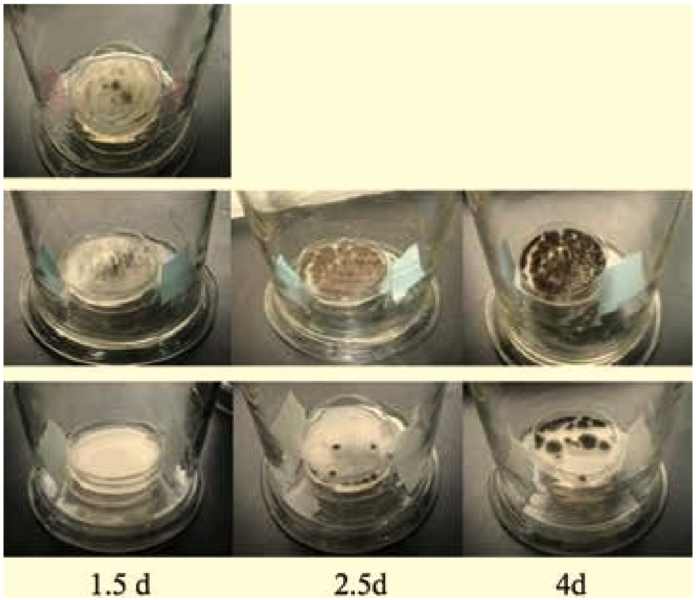

Figure 6. Plate pictures inoculated *Aspergillus niger* under the action of mildewproof sheets produced from SCG-II (white, bottom), MICROGARDE (blue, middle) and WASAOURO (red, top).[18]

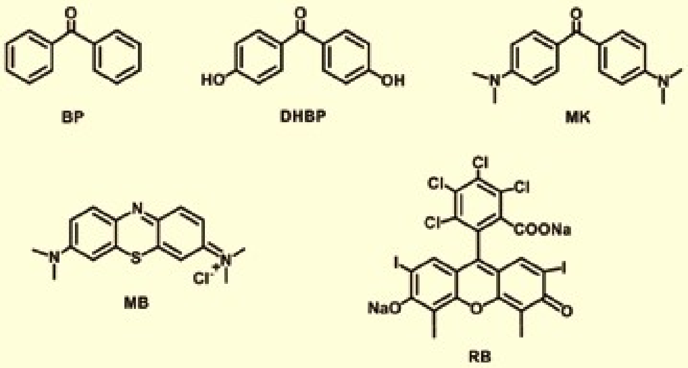

Figure 7. Photosensitizers used in leather finishing to prepare antimicrobial coating.[24,25]

As shown in Figure 7, photoactive agents such as benzophenone (BP), 4,4’-dihydroxybenzophenone (DHBP), 4,4’-bis(dimethylamino) benzophenone (MK), rose bengal (RB) and methylene blue (MB) could be used to prepare antimicrobial coating on leather surfaces with high potency against microorganisms by incorporating them into polyurethane (PU)-based coating solutions.[24,25] Under UVA irradiation, these photosensitizers generate free radicals in the triplet state by absorbing the effective hydrogen from the carbamate group in PU, and when exposed to oxygen, as shown in Figure 8, these radicals can be rapidly oxidized back to initial state with an accompanying formation of $H_2O_2$. Both the radicals and $H_2O_2$ provide antimicrobial functions. The surface-antimicrobial leathers were prepared by a painting method, and their antimicrobial ability showed the effective durability to abrasion and daylight.[24,25]

Chen and Fan *et al*.[26] yielded an enzymatically-switchable antimicrobial PU-coating for leather finishing by conjugating covalently biocide sulfanilamide (SA) into the PU backbone as chain extender. In the presence of urease, a representative hydrolase derived from one microbial species prevalent on leather coating, as shown in Figure 9, this PU coating was found to release free SA by urease-catalyzed cleavage of urea linkages. The regenerated SA molecules still exhibited antimicrobial efficiency as pristine ones. When no urease was in the environment, however, the coating displayed substantially high resistance against hydrolysis and maintained structural integrity. The enzymatically-responsive PU designed has great potential as an ideal leather-finishing material, which is capable of providing efficient protection against microbial deterioration while minimizing undesirable side effects associated with biocide abuse.

A synergetic combination of BHB and isothiazolinone was found by Chen *et al*.[27] for the development of antimicrobial leather insole for children. The antimicrobial effects of the prepared insole decreased gradually with the increasing times of washing and seat-soaking, but, after 20 times of washing or 10 times of seat-soaking, the insole still showed good antimicrobial properties that were confirmed by inhibition zone test. This leather insole is helpful for the control of pathogenic microorganism in children shoes. A series of research work were also done in their reports.[28-30]

## 3. Natural Essential Oils

To replace the synthesized antimicrobials with high toxic effects on human beings and environment, many efforts have been paid on the natural substances such as essential oils and chitosan derivatives. Based on their molecular weight, these natural antimicrobials can be divided into two types, namely, micromolecular and macromolecular ones. Essential oils, the natural mixtures of the secondary metabolites from plants, are the first type. Normally, their main components are small molecules such as terpenes, aromatic, aliphatic compounds, nitrogen- and sulfur-containing compounds.[31] Because of their eco-friendly property and diverse bioactivities, essential oils have been becoming increasingly popular and widely used in many different sectors including food industry, pharmacy, cosmetic, spice, health protection and textile industry.[31] In the leather industry, all kinds of essential oils with antibacterial or /and antifungal properties, as shown in Table II, have been investigated for their possibility to be used as antimicrobials to protect tanned leathers such as wet blues, crust leathers, finished leathers and their goods.

In 2006, Bayramoglu *et al.,*[32] for the first time, examined the applicability of *Origanum minutiflorum* (oregano) essential oil as a fungicide against fungi that grow on leather during the pickling and tanning processes. Oregano is an endemic species in Turkey, from which essential oil is produced from its leaves and flowering tops by steam distillation. Their main ingredients included carvacrol, $\gamma$-terpinene, *p*-cimetidine, and

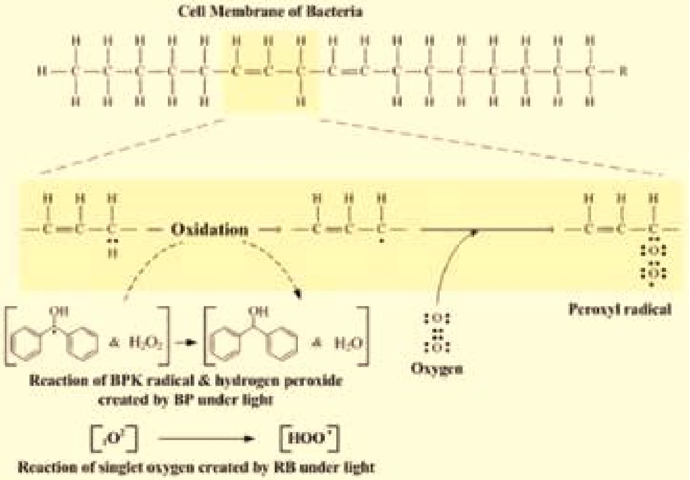

Figure 8. Potential peroxidation reaction involving the photoactive agents (BP and RB) and a fatty acid in cell membrane of bacteria.[24]

Figure 9. Schematic illustration of possible mechanism by which the SA-conjugated PU leather coating functions.[26]

β-caryophyllene. The optimum antifungal effect of oregano essential oil on leathers was obtained at 2% based on ASTM D 4576/86 standards, while the commercial fungicides (TCMTB and OITZ) used at suggested amounts did not provide enough protection. They concluded that *Origanum minutiflorum* essential oil can be used as a new fungicide that does not give harm to nature or human health. Besides, unlike pharmaceutical antibiotics, essential oils do not create resistant strains of mutant bacteria, and can prevent Cr(VI) formation in leather.[32] Bayramoglu *et al*.[33,34] also tested the applicability

## TABLE II
## Essential oils studied in leather industry.

| Essential oils | Main ingredients | Objects | Origin | Year | Ref. |
|---|---|---|---|---|---|
| *Origanum minutiflorum* | Carvacrol, γ-Terpinene, *p*-Cimetidine, β-Caryophyllene | Picking, Tanning | Turkey | 2006 | 32 |
| *Schinus molle* | α-Phellandrene, β-Phellandrene, Limonene, β-Myrcene, α-Pinene | Wet-blue | Turkey | 2008 | 33 |
| *Rosmarinus officinalis* | Rosmanol, Rosmarinic acid, Carnosol, 1,8-Cineole, Camphor, α-Pinene, β-Pinene, | Picking, Tanning | Turkey | 2010 | 34 |
| *Myrtus communis* | Linalool, Myrtenyl acetate, 1,8-Cineole, α-Terpineol, Geranyl acetate, α-Pinene | Picking, Tanning | Turkey | 2010 | 34 |
| *Clove* | 3-allylguaiacol, β-Caryophyllene | Wet-blue, Crust leather | China | 2011, 2013 | 31, 38, 39 |
| *Cinnamon* | (E)-Cinnamaldehyde | Wet-blue | China | 2011 | 31 |
| *Garlic* | Diallyl trisulfide, Methyl 2-propenyl trisulfide, | Wet-blue | China | 2011 | 31, 40 |
| *Star anise* | *p*-Anethole | Wet-blue | China | 2011 | 31 |
| *Aloe vera* | Aloin, Aloe-emodin | Split Suede leather | Turkey | 2010 | 35 |
| *Eucalyptus globulus* | 1,8-Cineole, D-Limonene, α-Pinene | Fatliquored leather | Lithuania, Ukraine | 2011 | 36 |
| *Lavandulae officinalis* | Linalyl acetate, Linalool, 1.8-Cineole, α-Pinene | Fatliquored leather | Lithuania, Ukraine | 2011 | 36 |
| *Thymus vulgaris*<br>*Thymus serpyllum* | Thymol, γ-Terpinene, β-Cymene, Carvacrol, 1.8-Cineole | Fatliquored leather | Lithuania | 2012 | 37 |
| *Melaleuca alternifolia* (Tea Tree) | Terpinen-4-ol, c-Terpinene, α-Terpiene, 1,8-Cineole | Footwear materials | Spain | 2011, 2012, 2014 | 41-43 |
| *Artemisia argyi* | 1,8-Cineol, Borneol, Camphor, Piperitol | Wet-blue, PU | China | 2007, 2012, 2013 | 44-49 |
| *Pseudevernia furfuracea* | Atraric acid, Olivetol, Olivetonide | Chrome tanned leather | Turkey | 2013 | 50 |
| *Lawsonia inermis* | Lawsone, 1,4-Naphthoquinone, Gallic acid | Fungi from finished leather | India | 2011 | 51 |
| *Mentha piperita* | Menthol, Menthone, 1,8-Cineole, Pulegone | Hide collagen | Turkey | 2013 | 52 |

of other essential oils such as *Origanum sp.*, *Schinus molle*, *Rosmarinus officinalis* and *Myrtus communis* as bactericides and fungicides against bacteria and fungi that grow on leathers during pickling and tanning processes.

In 2011, the possibilities of four essential oils extracted from traditional Chinese medicinal materials as leather fungicides were investigated by Gu *et al.*[31] The tested essential oils included cinnamon oil, garlic oil, clove oil and star anise oil, and a TCMTB-containing commercial fungicide was used as control to inhibit the growth of molds on wet-blue. It was found that these essential oils had antifungal activities and their effect improves with increasing concentration. In particular, for garlic oil, clove oil and cinnamon oil, the dosage of 2% was enough to inhibit the growth of all the tested molds on wet-blue whilst for star anise oil, more than 2% was required. These essential oils show different inhibitory effects on different molds, and in general, *Penicilliu citrinum* and *Alternaria alternate* are the species most sensitive to the essential oils, *Aspergillus niger* is rated second, and *Rhizopus stolonifer* is the most resistant one. Considering their advantages of being eco-friendly natural products and their acceptable economic cost, these essential oils are potential to be used as fungicides used in leather industry.[31]

Besides protecting wet blues, essential oils were tested as antimicrobials to prepare antimicrobial function leathers. For example, Bitlisli *et al.*[35] added a range of *Aloe vera* concentrations in the fat-liquoring process to treat split suede leathers that then showed antimicrobial properties against some Gram (-) and Gram (+) bacteria and *C. albicans*. Leathers treated with *Aloe vera* oil took a yellowish-brown color, and their moisture content and softness increased according to the concentration of *Aloe vera* used. In addition, the leathers that were treated with 6% or more *Aloe vera* had slightly higher tear load values when compared with control leathers.

Sirvaityte *et al.*[36,37] investigated the possibility of using essential oils including *Eucalyptus globulus*, *Lavandulae officinalis*, *Thymus vulgaris* and *Thymus serpyllum* as alternative preservatives for fat-liquored leather. These essential oils were added into the fat-liquoring emulsion, and the TCMTB-based fungicide was used as control. The tested microbial species contained *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The *Lavandulae officinalis* essential oil ensured better antibacterial effect than *Eucalyptus globulus* one. Gram-positive bacteria were found to be more sensitive to the essential oil of thyme than Gram-negative bacteria. As a main result of this study, it is concluded that the selected essential oils can be used as preservation agents in leather industry. After 4 weeks storage, the leather preserved with TCMTB-based fungicide have weaker protection comparing with the samples treated, respectively, with the amount of 5% (% on tanned leather mass) *Lavandulae officinalis* oil and less than 3% of thyme oil. Furthermore, the

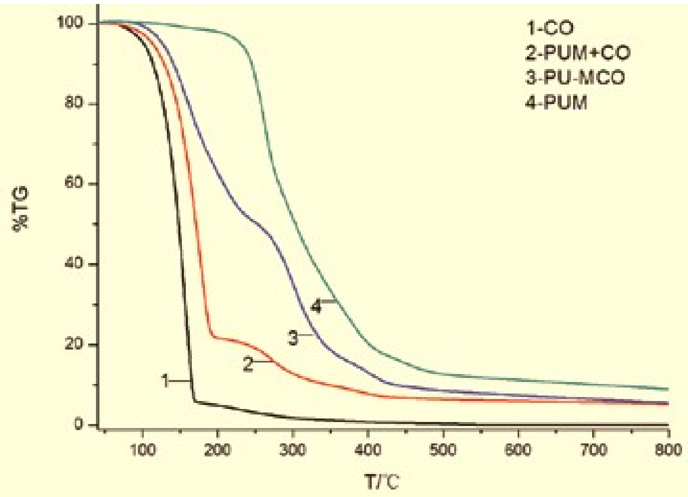

Figure 10. TG curves of CO, PU-MCO, PUM and PM+CO. [39]

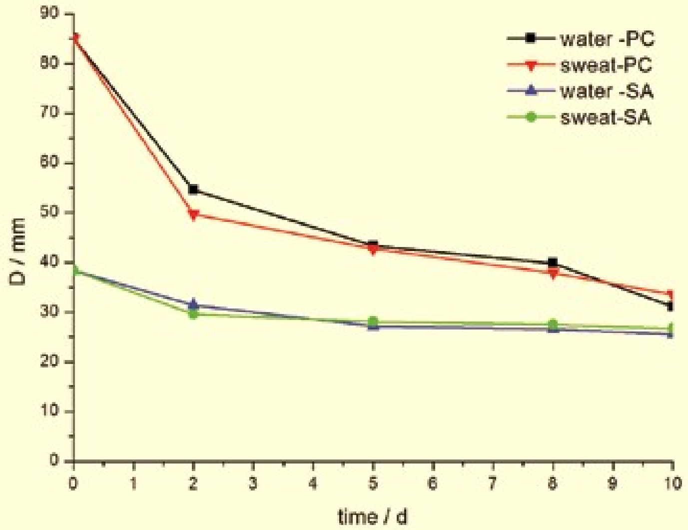

Figure 11. Antimicrobial activities of leather washed by water and sweat.[39]
Water-PC: water washing against Penicillium citrinum
Sweat-PC: sweat washing against Penicillium citrinum
Water-SA: water washing against Staphylococcus aureus
Sweat-SA: sweat washing against Staphylococcus aureus

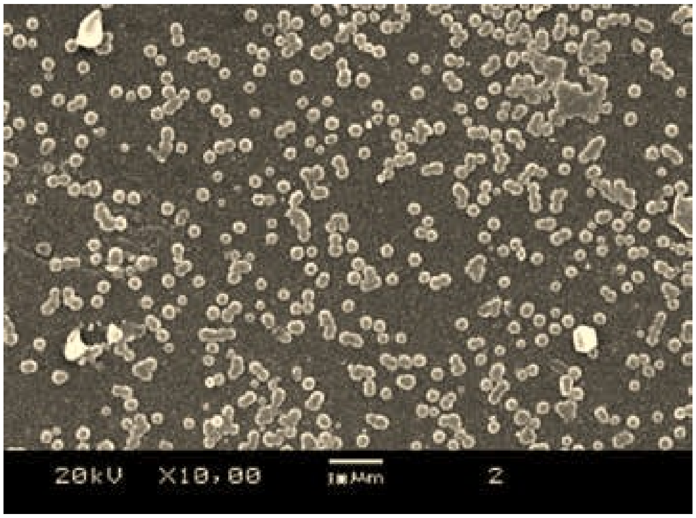

Figure 12. SEM photograph of PU microcapsules of garlic oil. [40]

essential oil of thyme can be used as a preservative in a mixture with a synthetic biocide containing TCMTB.[36,37]

However, natural essential oils have some shortcomings such as volatilization, instability and low water-solubility, and some of them have strong and offensive odors. To solve these problems, microcapsule technology was often used to improve their usability in leather industry. Microencapsulation can give essential oils improved physical and use properties such as better water-solubility and appearance. More importantly, active ingredients of essential oils can be made more stable because the outer wall material can prevent them from contact with adverse environmental factors such as light and oxygen, and their volatility can also be greatly reduced. In this respect, Gu et al.[38,39] ever adopted aqueous polyurethane (PU)”. as the wall material to microencapsulate natural clove oil by interface polymerization. The microencapsulation can significantly improve the thermal stability of clove oil, as shown in Figure 10, and afford it good controlled-release property. The PU-microencapsulated clove oil (PU-MCO) emulsion was then successfully applied in the post-tanning process to produce the functional antimicrobial goat garment leather that has the lasting ability to kill or inhibit harmful microorganisms. Notably, as shown in Figure 11, the leather obtained showed good antimicrobial property which persisted for a long time at room temperature and was not significantly affected by water and sweat washing because of the firm physical and chemical combination between the PU wall material and leather fibers. Similarly, Gu *et al*.[40] also prepared the PU microcapsules of garlic oil (Figure 12) by the interfacial polymerization method, and the goat wet blue treated by the microcapsule emulsion at the concentration of 0.5-1.5% garlic oil showed remarkable inhibitory effect against *Penicillium citrinum* and *Aspergillus niger.*

Also, the microcapsules of *Melaleuca alternifolia* (Tea Tree) oil was prepared and used as a natural biocide to develop footwear materials with antimicrobial properties.[41-43] In 2011, Sanchez-Navarro *et al*.[41] carried out the microencapsulation of this essential oil by *in situ* polymerization method using a melamine-formaldehyde resin as shell material in order to increase the durability of this natural biocide in footwear materials. The incorporation of the microencapsulated biocide into different footwear materials (leather and fabric) was achieved by immersing method, and the anchorage was confirmed by SEM as shown in Figure 13. For the same purpose, Perez-Liminana *et al*.[43] synthesized the gelatine-carboxymethylcellulose based microcapsules containing tea tree oil by a complex coacervation process. Meanwhile, the influence of the gelatine (G)/sodium carboxymethyl-methyl cellulose (CMC) ratio (G/C) on the microcapsule properties, as well as the microencapsulation oil efficiency, was evaluated. The microcapsule durability under different conditions, such as rubbing and ironing, was analyzed in order to simulate shoe manufacturing and shoe wearing.

Furthermore, the microencapsulation of *Artemisia argyi* essential oil attracted the attention of many leather chemists because of its excellent antimicrobial properties. Especially, Wang’s group[44-48] adopted various shell materials and polymerization techniques to prepare spherical microcapsules of *Artemisia argyi* essential oil with improved thermostability and certain controlled-releasing function, and the treated wet blue and PU showed obvious inhibitory effect against bacteria and fungi. In addition, Hu *et al*.[49] prepared the *Artemisia argyi* oil (AAO)-loaded antibacterial microcapsules with hydroxyapatite (HAp)/poly (melamine formaldehyde) (PMF) hybrid shells by self-assembly of HAp nanoparticles at the interface of O/W emulsions and subsequent *in situ* polymerization of melamine formaldehyde pre-polymer. The prepared microcapsules had a spherical shape and rough surface, and the thermal stability of AAO in the microcapsules was higher than that of unpacked AAO. The release profiles of AAO from the microcapsules followed Higuchi kinetic model and release rate increased as the temperature rising. Moreover, the microcapsules had a long-term antimicrobial effect with bacterial inhibition rate against *S. aureus* and *E. coli* maintained as high as 83% even after storage for 60 days.

Other essential oils such as *Pseudevernia furfuracea*, *Lawsonia inermis* and *Mentha piperita* were also investigated for the antimicrobial application in leather industry.[50-54]

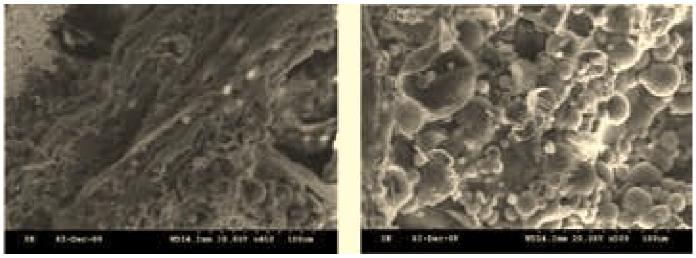

Figure 13. Tea tree oil microcapsules incorporated to leather used as footwear material.[41]

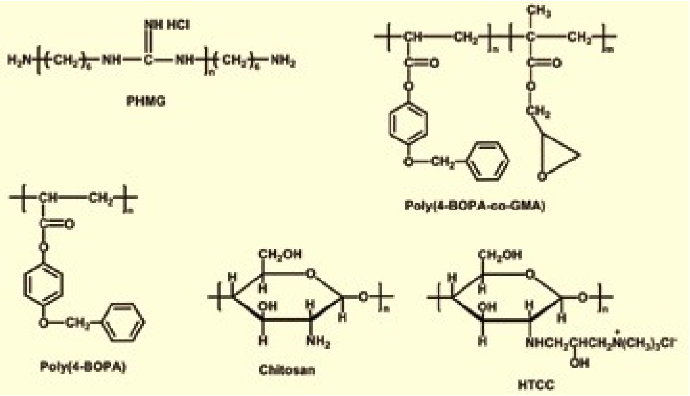

Figure 14. Some macromolecular antimicrobials investigated in leather industry.

## 4. Macromolecular Antimicrobials

Antimicrobials with high molecular weight were increasingly noticed in leather industry due to their lower toxicity compared with small-molecule ones. In general, they can be classified into synthetic and natural types according to their origin. Antimicrobial polymers are good samples for the former, while chitosan is the representative one for the latter.

### *4.1 Antimicrobial Polymers*

There are sporadic reports[55-57] about the investigation of synthetic antimicrobial polymers used in leather industry. For example, in 2011, Huang *et al.*[55] investigated the application of polyhexamethylene guanidine hydrochloride (PHMG) in tanning industry. PHMG presented highly inhibitory effect against most of the bacteria found in leather-making process, such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Heterotrophic bacteria* and so on. With a dosage of 30 mg/kg, the ratio of sterilization can reached more than 90%. When used in the wet end process, PHMG can give leather good bactericidal effect. Furthermore, Subramanian et al [56] synthesized the antibacterial poly(4-benzyloxyphenylacrylate) and its copolymers with glycidyl methacrylate (Figure 14), which showed good adhesive strength for leather, and thus can be used to prepare antibacterial coating.

### *4.2 Chitosan Derivatives*

Although chitosan and its derivatives have been known for their nice antimicrobial activities for decades, their applications as biocides in leather industry were investigated only recently. In 2006, Lu *et al.* [58] carried out firstly the antibacterial application of chitosan in leather making process. The commercial chitosan was added into the fixing bath of fat-liquors, which is the last step of post-tanning wet processing to prepare the split pig leather as shoe lining. According to the optimal experiment about the process factors, it was found that the obtained leather showed more than 80% of inhibitory ratio against *Staphylococcus aureus* (ATTCC 6538) when the process condition was as follows: 0.45% of chitosan (% based on wet-blue weight), pH 2.8-3.0, 60°C and 60-90 min. Besides, Fernandes *et al.*[59] also obtained positive results when they developed antimicrobial leather insoles by taking advantage of chitosan's intrinsic antimicrobial activity and film forming capacity. Two main approaches were assayed, namely, an integrated approach corresponding to an impregnation performed as an additional step after the dying stage (tested using a pilot scale drum), and a post-treatment approach where the coating is applied as a finishing step either using a spray-gun or a calender. It was found that chitosan coating conferred antimicrobial properties to the treated leathers, and the higher capacity to eliminate *E. coli* was achieved when the drum technology is used. The advantage of this approach is that the dye fixation stage already comprises the use of acidic solutions, which simplifies the introduction of this new stage (addition of chitosan solution) in the whole process. Considering both antimicrobial efficacy and economic benefits, coating using 1% chitosan content (formic acid solution) performed in the drum during 2 h, was proposed as the best solution to be used by the tanning industry.

As shown in Figure 14, N-(2-hydroxypropyl-3-trimethyl ammonium) chitosan chloride (HTCC), which has better water-solubility and antimicrobial property than chitosan, was also investigated for the antimicrobial application in leather industry. Likewise, the first application of HTCC was conducted by Lu *et al.*[60] in 2006. Like the chitosan, the HTCC was used to treat split pig shoe-lining leather in the final process of post-tanning wet processing. Notably, the pH change from 2.5-4.0 had hardly effect on the antibacterial property of the obtained leather, and the inhibitory rate against *Staphylococcus aureus* reached more than 91% when 0.4% of chitosan (% based on wet-blue weight) was added, which further confirmed the better antibacterial activity and usability of HTCC. Recently, Dan *et al.*[61] indicated antifungal effect of HTCC in wet-blue cattle hides. The optimized antifungal process was a float of 150% with dosage of 0.2% at pH 5.0, below 40°C, reacting for 2 hours. In addition, other antimicrobial derivatives, such as the graft copolymer of degraded chitosan with methacrylic acid (MAA) and acrylamide (AAM),[62] carboxy-methylated chitosan with chloroacetic acid,[63] and so on, were reported and applied in the leather-making process to confer antimicrobial capacity.

## 5. Nano-inorganic Antimicrobials

Antimicrobial nanomaterials are receiving more and more attention in leather industry because of their excellent antimicrobial activities and biological safety.[64-66] As ecological alternatives to organic antimicrobials currently used, they can be used to prepare high value-added leathers with antimicrobial surfaces or antimicrobial function leathers where nanoparticles deposited not only on the grain and flesh sides, but also into collagen fibrillar structure. In general, there are following methods by which the nano-inorganic antimicrobials were introduced into leather substrate. (1) Antimicrobial nanoparticles were directly dispersed into finishing agents, and then coated on the leather grain sides to prepare antimicrobial surface.[67-69] (2) The nanoparticle/polymer composites, which were synthesized by *in-situ* polymerization in the presence of inorganic nanostructures or precursors, was used as finishing agents to get antimicrobial leather surface.[70-73] (3) Immersing or drum-processing in leather-making process such as tanning, retanning and fat-liquoring were adopted to deposit nanoparticles onto hide fibers and surfaces, which resulted in antimicrobial wet-blue, crust leather, finished leather and fur.[74-86] (4) Tannage based on nanomaterials, especially nano-$SiO_2$, were developed to prepare leather with antimicrobial properties.[87-89]

In the leather industry, much interest was directed to antimicrobial nanosilver,[74-85,90-93] nano-ZnO,[71,72, 94-96] nano-$TiO_{2,}$[67,69,70,73,97] nano-$SiO_{2,}$[68,87-89, 98-103] nano-copper,[86] and so on.

### *5.1 Nanosilver*

Among various antimicrobial nanomaterials, nanosilver is the most promising one, which has a broad-spectrum antimicrobial activity to kill a variety of bacteria and fungi existing in everyday life, nosocomial environments and industrial processes. In the past few years, because of their excellent biological safety, silver nanoparticles were adopted to treat leathers and furs for medical use. The medical products are well known for preventive and curative properties, suitable for treatment of orthopedic, diabetic or bedsore diseases. In this filed, the efforts done by Gaidau *et al*.[74-80] are notable. Using electrochemical or chemical methods, they synthesized colloidal silver solutions (CSS), and especially, the Ag/$TiO_2$ nano-dispersed system was also prepared by electrochemically covering $TiO_2$ nanoparticles with silver nanoparticles. Because of its excellent structure, properties such as high surface areas and continuous pore structure, mesoporous $TiO_2$ is an excellent support material of silver nanoparticles. Using CSS with or without $TiO_2$, the treatment of leathers, including wet blue, metal-free crust leather and medical sheepskins, was carried out by immersion, by spraying, in tanning bath, in retanning or neutralization bath. As shown in Figure 15, AgNPs deposition onto collagen and keratin structure of sheepskins was certified by SEM technique. The presence of AgNPs both deep inside the sheepskin structure and onto its surface (including the keratin fibers as well) is a valuable indication of an adequate treatment performed upon sheepskin. The resistance of AgNPs treated sheepskins to fungi and bacteria was evaluated by antibiogram method, diffusimetric method and standard methods (ASTM D 4576-86) for leather materials. Very good resistance to fungi was observed in the case of treated leathers and wool with the AgNPs-based system as can be seen in Figure 16 and 17, and testing the biocidal effect upon *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 9027) indicated excellent bactericidal action against two of the strains specific for the hospital environment, with high resistance to many bactericides. In addition, the influence of sheepskins treated with silver nanoparticles on the wound healing process was assessed, and the nanoparticles concentration seems to have a positive effect up to 370 ppm and does not influence the inflammatory process above this concentration.

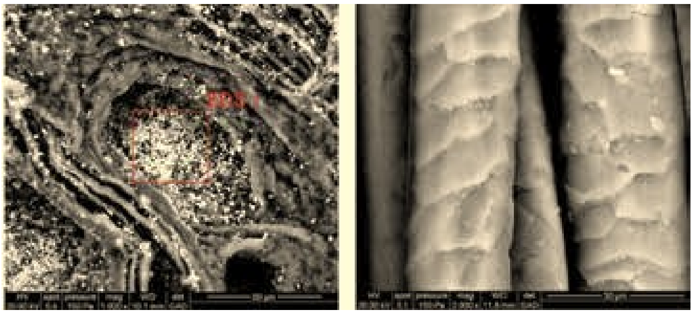

Figure 15. SEM images of AgNPs deposited onto collagen fibrillar structure of dermal layer (left) and keratin fibers (right) of sheepskin.[79]

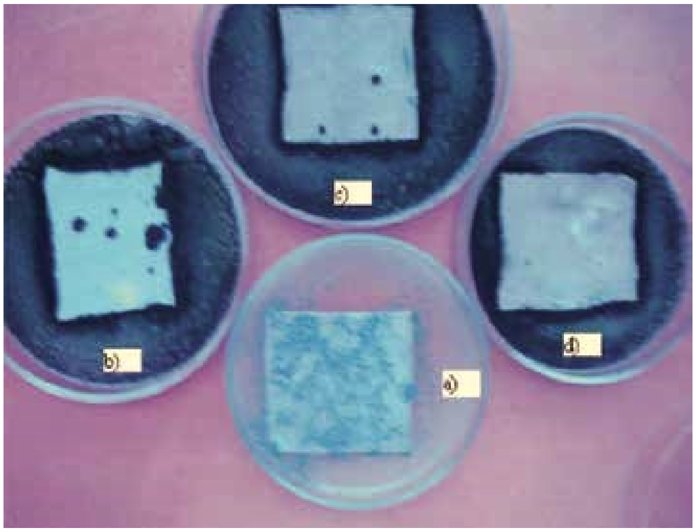

Figure 16. Fungitoxic effect on leather support expressed by mold growth: a) witness, b) 32 ppm Ag CSS, c) Ag/$TiO_2$ CSS with 10 g/l $TiO_2$, d) Ag/$TiO_2$ CSS with 50 g/l $TiO_2$, after 7 days.[74]

Many efforts have been paid by Chen's group[81-85,90] on the preparation of antimicrobial leathers and furs, too. Using a cationic surfactant benzalkonium bromide as the dispersant, silver nanoparticles were synthesized and applied to produce

Figure 17. The sheepskin treated with AgNPs (left) and without treatment (right) after 14 days of fungi exposure.[79]

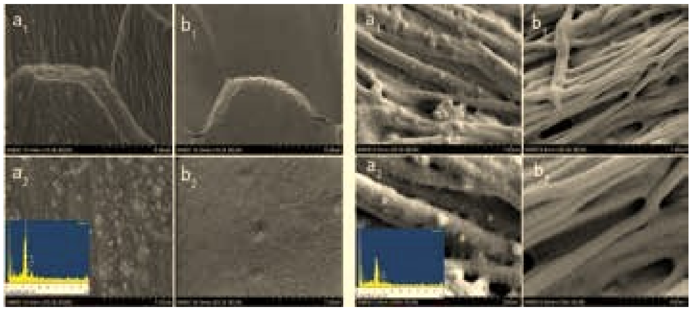

Figure 18. SEM images of the wool (left) and leather (right) with silver nanoparticles ($a_1$, $a_2$) and control ($b_1$, $b_2$). EDS analysis shows peaks of silver (small panel).[81]

the antibacterial sheepskin that has the potential use of pressure ulcer prevention for elderly people residing in nursing home and patients perennially lying in bed.[81,90] The glutaraldehyde tanned sheepskin was immersed in the nanosilver solution with different concentration, and shaken at 30˚C for 3 hours. The UV-vis absorption spectroscopy indicated nearly all of the silver nanoparticles were absorbed by the sheepskin in this way. As shown in Figure 18, silver nanoparticles attached on the surface of wool and collagen fibrils. The treated sheepskin with $2.4 \times 10^{-2}$ g/L of nanosilver had an antibacterial inhibition of 99.9% against *E. coli* and *S. aureus*, and even after 6 cycles of perspiration treatment, the sheepskin still exhibited a durable antibacterial effect with the inhibition rate of 79.4% and 67.1% respective to the leather and wool.[81] Based on above research results, they further developed the manufacturing technology of antibacterial sheep fur at industrial scale under the requirement of ecological leather, in which the chrome tanning was strengthened and the nanosilver antibacterial treatment was carried out at the final stage of post-tanning wet process.[82,83] Furthermore, the nanosilver-containing antimicrobial agent was also applied by surface-coating technique and simple spraying method, respectively, to prepare bovine hide sleeping mat leather and shoe lining leather with antibacterial and antifungal properties.[84,85]

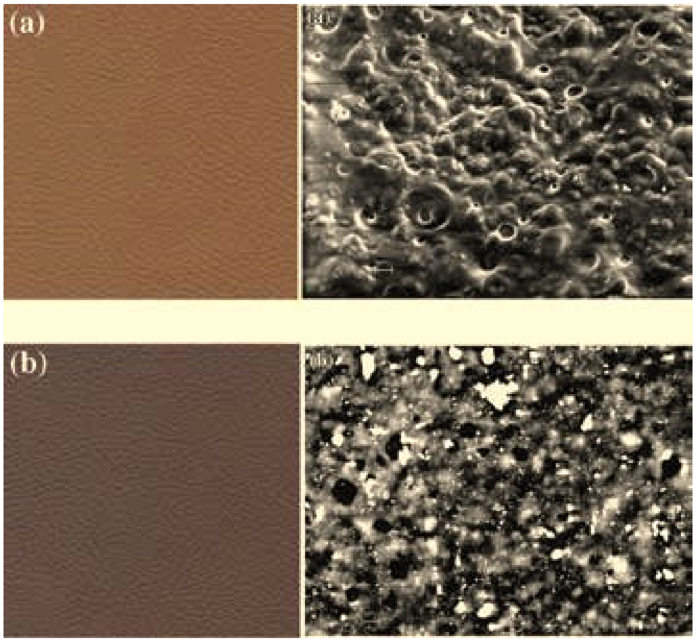

Figure 19. Visual and SEM comparison of untreated (a) and nanosilver-treated (b) natural leather.[91]

Pollini *et al*.[91] prepared antibacterial natural leather for application in the public transport system using an innovative silver deposition technology. The technology was based on the *in situ* photoreduction (365 nm of ultraviolet irradiation for 5

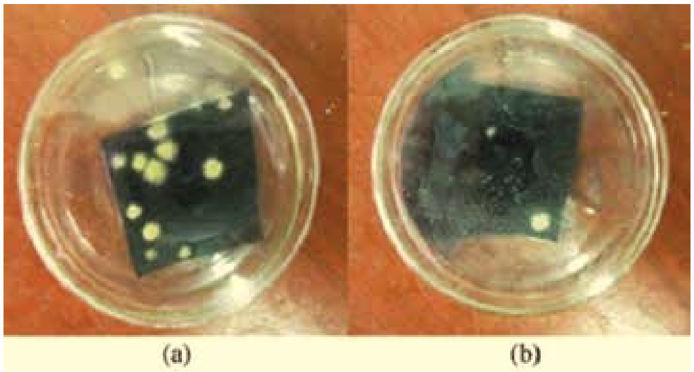

Figure 20. Antimicrobial behavior of leather finished by (a) polyacrylate and (b) polyacrylate/ZnO nanocomposites.[71]

## TABLE III
## Zone inhibitions of polyacrylate film and polyacrylate/ZnO nanocomposite films with ZnO nanoparticles of different morphologies.[72]

| **Sample (ZnO contents)** | **D (mm)** *Aspergillus flavus* **0.5%** | **D (mm)** *Aspergillus flavus* **1.0%** | **D (mm)** *Aspergillus flavus* **5%** | **D (mm)** *Candida albicans* **0.5%** | **D (mm)** *Candida albicans* **1.0%** | **D (mm)** *Candida albicans* **5%** |
|---|---|---|---|---|---|---|
| Polyacrylate film | 0 | 0 | 0 | 0 | 0 | 0 |
| Film containing sphere-like ZnO | 0.5 | 1.8 | 3.2 | 2.5 | 5.0 | 8.2 |
| Film containing flower-like ZnO | 0.8 | 2.0 | 3.0 | 4.5 | 4.8 | 8.0 |
| Film containing rod-like ZnO | 0.4 | 1.2 | 2.0 | 2.5 | 4.0 | 4.0 |
| Film containing sheet-like ZnO | 0 | 0 | 0 | 1.8 | 3.2 | 6.4 |
| Film containing needle-like ZnO | 0 | 0 | 1.5 | 1.2 | 3.2 | 5.5 |

min) of a silver solution directly sprayed on the surface of the natural finished leather with a thin polyurethane film (35 μm). As shown in Figure 19, the comparison between untreated and silver treated natural leather was characterized by an evident change in color due to the formation of silver clusters uniformly distributed on the treated substrate. An impressive antibacterial capability against Gram-negative and Gram-positive bacteria was demonstrated after the Taber abrasion tests, suggesting a strong adhesion of the silver clusters to the substrate that preserves its efficacy despite the condition of use. Moreover, the process does not require expensive equipment and involves the only surface of the material, thus reducing the amount of silver solution needed. So, the presented technology is a promising instrument to contain the contamination in public transport vehicles.

Velmurugan *et al.*[92] investigated the antimicrobial fabrication of tanned leather using green-synthesized nanosilver by *Erigeron annuus* (L.) pers flower extract as reducing and capping agent. With the help of ultrasonicator, the nanosilver materials were embedded to the leather without smooth surface by immersing method. It is found that combination of flower extract with AgNPs is more enough to exhibit excellent antibacterial activity against Gram-positive odor causing bacteria *Brevibacterium linens* and *Staphylococcus epidermidis*, and the flower extract might play as a binder for nanoparticles, which showed maximum antibacterial efficiency of the treated leather. Similar research results were also founded when nanoparticles were prepared by autoclaving the silver ion with the pine gum acting as reducing and capping agent.[93]

### *5.2 Nano-ZnO*

Because of its ideal filling property, high stability, excellent antibacterial and non-toxic behavior, nano-ZnO material has attracted widespread attention in leather industry.[71,72, 94-96] In 2008, Chen *et al.*[94] prepared antibacterial and antifungal collagen-ZnO nanocomposite using the sol-gel process. The precursor of nano-ZnO, which was synthesized by zinc acetate, ammonium citrate and ethanol, was used to treat collagen fibers cross-linked by glutaraldehyde in drum at 50˚C for 9 hours. Nano-ZnO with 25 nm particle size formed *in-situ* and deposited on collagen fibers, which resulted in the formation of collagen-ZnO nanocomposite. The introduction of nano-ZnO endowed the collagen-based material with obvious antibacterial and mildew-proof ability. Furthermore, the commercial nano-ZnO with 25-35 nm particle size was used to prepare children shoes insole with microbial resistance function.[95] The ultrasonication was adopted to accelerate the immersion of insole leather in the emulsion containing nano-ZnO. The obtained products showed more than 97% of inhibition rate against representative bacteria, yeasts and molds isolated from children shoes, and even after 10 times of water-washing treatment or 6 cycles of perspiration immersion, more than 92% of inhibit rates were observed, which indicated the durable antimicrobial effect of the obtained insole leathers.[95]

Ma's group[71] developed polyacrylate/ZnO nanocomposite for leather finishing by employing anionic polymer (PA30) as the surface modification agent via *in situ* emulsion polymerization. PA30 was grafted to the ZnO surface, and the PA30 modified ZnO particles, which showed much improved stability and dispersibility than those of unmodified ZnO particles, can be dispersed in polyacrylate film rather homogeneously. Leather finished with polyacrylate/ZnO nanocomposites exhibited not only stronger inhibitory activity against *Aspergillus flavus* (Figure 20), but also favorable sanitation properties, where the vapor permeability and water-vapor permeability were improved by 164.72% and 114.3% respectively, compared with those of leather finished with polyacrylate. Meanwhile, the nanoparticle morphology and film-forming behavior of polyacrylate/ZnO nanocomposite were also reported by them.[72] Film-forming agents of polyacrylate/ZnO nanocomposite, are formed by *in situ* emulsion polymerization of acrylate monomers in the presence of ZnO nanostructures with various morphologies including sphere-like, rod-like, sheet-like, needle-like and flower-like. It was found (Table III) that the films containing sphere-like ZnO and flower-like ZnO nanoparticles show excellent antibacterial activities, better than those of the films containing ZnO nanoparticles of other morphologies. With the addition of flower-like ZnO nanoparticles, water-vapor permeability of polyacrylate was increased by 122.17%. In contrast, the sphere-like ZnO nanoparticles gave rise to improvement in mechanical properties.[72]

### *5.3 Nano-TiO$_2$*

Many reports [69,70,73,97] investigated the application of Nano-TiO$_2$ in leather industry, where the antimicrobial collagen-TiO$_2$ nanocomposite or leather coating was developed. In 2006, Chen *et al* [97] reported the collagen-TiO$_2$ nanocomposite that was prepared by a sol-gel process procedure in which the picked goatskin was treated with the precursor solution of nano-TiO$_2$ and then glutaraldehyde. The precursor solution of nano-TiO$_2$ was composed of titanate Ti(OC$_4$H$_9$)$_4$, ethanol, acetic acid and water. It was demonstrated that, as shown in Table IV, the introduction of nano-TiO$_2$ endowed the leather with mold-proof capacity, and moreover, the shrinkage temperature of the nanocomposite could be improved from 73.8˚C to 82.2˚C by adding 6% glutaraldehyde which formed a cross-linkage between nano-TiO$_2$ and collagen fibrils.

By incorporating organic-inorganic nano-hybridization into wet phase inversion coating-forming method, Chen and Fan *et al.*[70] prepared a novel antimicrobial polyurethane synthetic leather coating with *in-situ* generated nano-TiO$_2$ (PUT). It was found that the antimicrobial activity of PUT coating increased with increasing nano-TiO$_2$ concentration, and when the nano-

$TiO_2$ concentration increased up to 0.75 and 1.00 wt%, the antibacterial activity of PUT coating exceeded 82% and 93% respectively, and no *Aspergillus niger* growth was observed on the coating surface within 28 days. Cell culture assay indicated that the PUT coating had no detrimental effect on the morphologies and proliferation rate of normal human dermal fibroblasts, which indicated a non-toxic and skin-friendly characteristic. In addition, Bao and Ma *et al*[73] recently prepared the polyacrylate/nano-$TiO_2$ composite latex via double *in-situ* polymerization using acrylamide (AM), vinyl acetate (VAc), Bu acrylate (BA) and Me methacrylate (MMA) as monomers, and tetra-Bu titanate ($Ti(OBu)_4$) as precursor of $TiO_2$, and the obtained composite latex was applied in leather finishing to prepare antibacterial coating with improved thermal stability and anti-yellowing property.

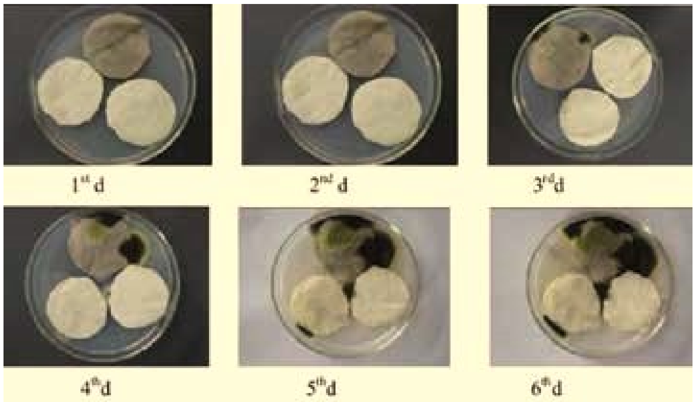

Figure 21. Photograph of the antifungal effect of nano-$SiO_2$ tanned (white) and chrome tanned (grey) leathers. [88]

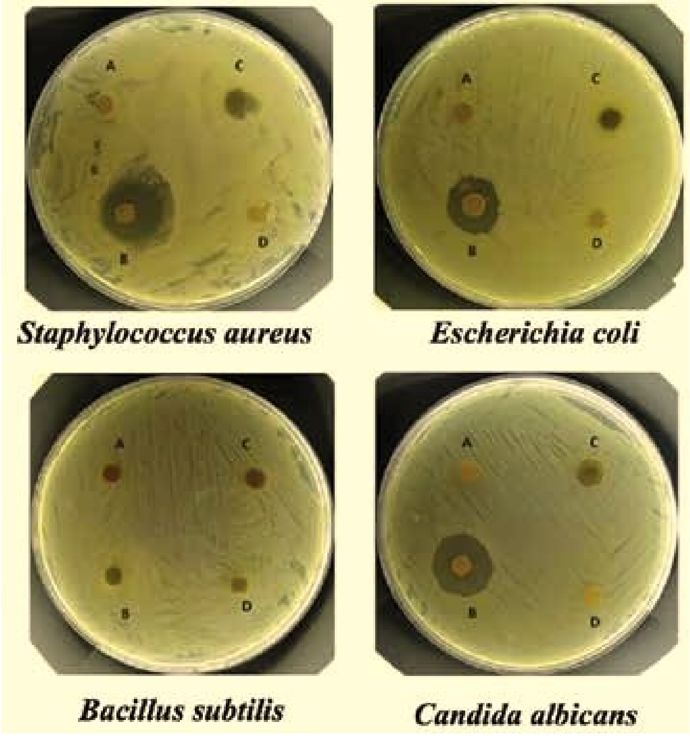

Figure 22. Photos of inhibition zones caused by calf crust leather disks treated with Cu nanoparticles suspension only once (C), twice (B) and the positive control solution (A and D). [86]

### *5.4 Nano-$SiO_2$*

Fan *et al*.[87-89, 98-102] have done remarkable work on the pickle-less nano-$SiO_2$ tannage in which a polymer or modified oil was used as dispersion supporter, and a precursor which can, *in-situ*, produce nano-$SiO_2$ under particular triggering condition was introduced into leather fibers. During the hydrolysis of precursor, nano-$SiO_2$ particles formed and reacted rapidly with the active groups of collagen. They proved that two new chemical bonds arose during the formation of the organic-inorganic nano-hydrid. One appeared to be the reaction between nano-$SiO_2$ formed *in-situ* by the hydrolysis of tetraethoxy silane and the -C=N- groups of arginine, histidine, and tryptophane in the backbone of collagen. In a second bond, occurring at the same time as the condensation of silanol proceeds, the pendent hydroxyl groups of collagen may partly reacted with Si-OH and formed a strong interaction between the organic and inorganic phases. These two chemical bonds seem to contribute to the leather's high hydrothermal stability. When the bated pelt is treated with 0.3 wt % nano-$SiO_2$ (based on bated pelt weight), the shrinkage temperature reached above 95°C. Compared to chrome tannage, leather tanned with oxazolidine-nano-$SiO_2$ demonstrated a higher resistance to mold than conventional wet-blue. As shown in Figure 21, the latter incubated for 3 days began to grow mildew, whilst the oxazolidine-nano-$SiO_2$ tanned leather which was incubated for 6 days had no mildew, showing a fine antifungal effect.[88]

Furthermore, there are reports about the antimicrobial application of nano-$SiO_2$ in leather finishing[68] and shoe materials.[103]

## TABLE IV
## The diameters of mold inhibition zones of samples (mm).[97]

| Sample | 1# | 2# | 3# |
|---|---|---|---|
| 1 | O | 18.96 | × |
| 2 | O | 19.38 | × |
| 3 | O | 19.38 | × |
| Average | O | 19.24 | × |

1# - nanoprecursor + glutaraldehyde; 2# - nanoprecursor/ no glutaraldehyde; 3# - glutaraldehyde only; O - no inhibition zone, but no mold growth on sample; × - no inhibition zone, mold growth on sample

### *5.5 Nano-copper*

For the first time, Galletti *et al.*[86] synthesized copper nanoparticles with 7-15 nm of average sizes under microwave (MW) irradiation in the absence of any stabilizing agent, and the obtained colloidal suspension was used to treat calf crust leather via impregnating method. As shown in Figure 22 and Table V, these ascertained data indicate that copper nanoparticles exert promising antibacterial activity against the tested bacteria, except for *Candida albicans*, and can be used for leather treatment. Moreover, this treated leather showed encouraging antistatic behavior.

## 6. Concluding Remarks

Normally, the troublesome microorganisms growing on tanned leathers are fungi, especially molds including *Penicillium*, *Aspergillus*, *Paecilomyces*, *Rhizopus* and *Mucor*, and the traditional processing objects include wet blue, crust leather, finished leather and leather goods.[2] In fact, frequently-used leather fungicides can meet requirements if the protection of leather's quality is the only considered. The main problem often worried is their toxicities to human beings and environment, and that is exactly the reason why leather researchers focused on the development of new fungicides. In this respect, following points are proposed to be considered:

(1) Much more attention should be paid to combined leather fungicides containing two or more synergetic active ingredients with low/no-toxicity. Although synergetic combinations often were reported, the corresponding industrial fungicide products rarely appeared, probably because of technology secrecy. Whatever, comprehensive study should be carried out on their formulation development, application method, industrial experiments toxicity and environmental impact assessment, etc.

(2) Natural essential oils are highly potential to be taken as alternatives of synthesized leather fungicides, but some of their shortcomings must be overcome. For example, the volatility and instability of essential oils can hinder their durability as leather fungicides. Although microencapsulation technique is feasible to solve this problem, considering the characteristics of leather matrix and its processing technology, many efforts should be focused on the choice of wall material, polymerization methods, compatibility to leather chemicals, multifunction, and so on. Furthermore, the contents and ratios of active ingredients can change with origin of corresponding plants, season, extracted parts and methods, which will directly result in the fluctuation of essential oil's quality. And another problem is the exorbitant prices for some essential oils, and they would not be economical when a big dosage is required. In this respect, combination technique is proposed to be a viable method to improve the antimicrobial properties of the final commercial products containing essential oils and lower their prices. Notably, the toxicity and environmental impact of essential oils should be assessed, too, as they are just mixtures of many chemical substances. Unpleasant smells of

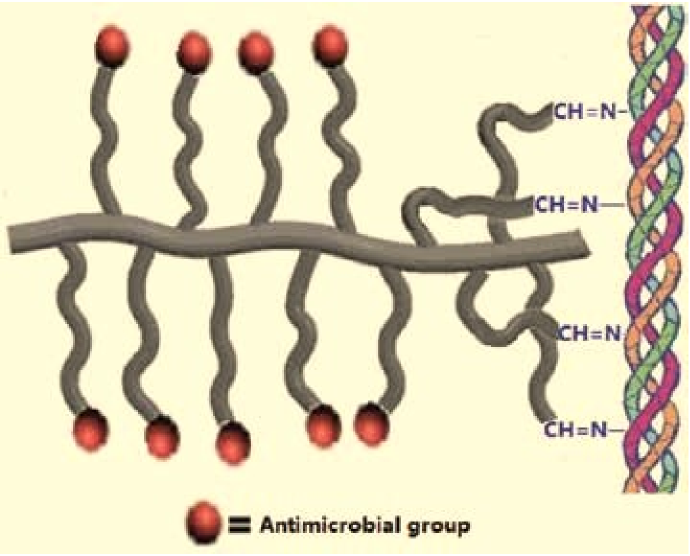

Figure 23. Possible binding mode between antimicrobial polymer containing aldehyde groups and collagen fibers.

**TABLE V**
**Antibacterial results of inhibition zones caused by calf crust leather disks treated with Cu nanoparticles suspension twice (B) and the positive control solution.[86]**

| Treatment of leather disk | *S. aureus* | | *E. coli* | | *B. subtilis* | | *C. albicans* | |
|---|---|---|---|---|---|---|---|---|
| | mm | E | mm | E | mm | E | mm | E |
| Double treatment with Cu suspension | 5.55 | ++++ | 2.60 | ++ | 2.80 | ++ | 0 | – |
| Positive control solution | 0.50 | ++ | 0 | – | 0 | + | 0 | – |

Estimation (E) for the diameters of inhibition zones: –, absent; +, <1 mm; ++, ≥1 mm and <2 mm; +++, ≥2 mm and <5 mm; ++++, ≥5 mm.

some essential oils also are a big hindrance for their application in leather, and the addition of essences and flavors perhaps is a feasible solution. In short, many efforts should be paid for the commercial scale application of essential oils in the control of microorganisms growing on tanned leathers.

(3) More systematical work is suggested to be carried out for the antimicrobial application of chitosan and its derivatives in leather industry. Frankly, unlike the typical leather fungicide such as TCMTB, chitosan products can not provide enough antifungal protection for leathers. So, the improvement of antimicrobial effect is the first task if chitosan and its derivatives are used as leather fungicides.

The research and development of antimicrobial function leather is still in initial stage, although an increasing number of recent reports have been focused on this field. As a burgeoning new area of study, antimicrobial functions for leather are faced with some theoretical and technical problems yet to be solved. For example, compared with the common leather fungicides, the used antimicrobial agents are required to have some special properties such as less toxicity, broader antimicrobial spectra and greater stability.[39] More importantly, the antimicrobial substances must be fixed on the leather surface or fibers, which can give leather with long-term antimicrobial performance. Finishing materials, especially the adhesive PU and acrylic resins, are good binders to immobilize antimicrobials on the leather coatings. However, to prepare antimicrobial leather in which antimicrobial compounds are distributed into leather fibers, how can their combination be carried out? In this respect, tanning chemistry maybe can give us some ideas. A good example is the nano-$SiO_2$ tannage presented by Fan *et al*.[100] in which two new chemical bonds were found between the nano-$SiO_2$ *in-situ* formed and reactive groups of collagen. Noticeably, the tanning effect of the polymer or modified oil, which was used as dispersion supporter, should be the more important factor that can result in the combination between the nano-$SiO_2$ and leather fibers.

PU-microencapsulated essential oil prepared by our group[39,40] is another example in which the fixation of essential oil onto leather fibers was based on tanning mechanism of PU. Due to the intensive physical and chemical interactions between leather fibers and PU that can be used as retanning and filling agents, the microencapsulated essential oil was firmly incorporated with collagen fibers and then can not easily be removed by the action of water, sweat and friction. Obviously, other antimicrobials with small molecule can also be fixed onto leather fibers by this method.

To sum up, two ways can be used to fix antimicrobials onto leather fibers via tanning mechanisms. The fixation of antimicrobial substance without tanning property can be carried out by the tanning effect of its carrier. Another feasible route may be the design of antimicrobial molecules with tanning effect. For instance, tanning groups and antimicrobial units can be connected to chitosan backbone by appropriate chemical modification. The former can promote the interaction between the chitosan molecule and collagen, while the latter can improve its antimicrobial activity. Similarly, antimicrobial polymers, which are rarely focused on by leather researchers, are also potential to be used to prepare antimicrobial function leather if they are endowed with tanning effect. These antimicrobial macromolecules may be introduced into leather fibers in the tanning or retanning process, and their binding mode can be simply illustrated by Figure 23 in which the aldehyde group is taken as a example of tanning groups. The tannage based on antimicrobial macromolecules may be a new chrome-free tanning approach.

In addition, it is difficult to compare the antimicrobial performance of antimicrobial function leathers reported in different literatures because of their various evaluation methods and conditions employed. In leather industry, there is now no clear and unambiguous definition about the antimicrobial function leather, let alone the standard evaluation method. And so, the authorized authentication and criterion should be established as soon as possible to meet the growing demand from leather consumers. Further, the antimicrobial durability is supposed to be considered, especially the action of washing, perspiration and abrasion on the antimicrobial performance of leathers.

In conclusion, leather exhibiting antimicrobial performance, as a function material, can be produced by various kinds of process and technique. Although many urgent problems need to be solved, the progress in this field will certainly promote the research and development of high value-added function leathers, and even may bring about a revolution in the leather-making technology.

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