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科研費 KAKENHI

# 科学研究費助成事業　　研究成果報告書

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研究課題名（英文）Bio-friendly nanosheets loaded with laser-generated nano-containers that release metal ions to accelerate wound healing

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研究成果の概要（和文）：ナノ粒子を組み込んだ生分解性ポリマーの薄いナノシートをこの研究で製造しました。これを応用することにより，皮膚と接触すると、生分解性ポリマー材料は、創傷治癒を促進する適切な金属イオンを放出すると考えられます。我々は最初に亜鉛、鉄、銅、マグネシウムのイオンを含むナノ粒子を製造しました。次いで、ナノ粒子を創傷被覆材として使用されるポリマーナノシートに組み込みました。
次に、製造したナノシートが人体温度で生理食塩水と接触したらどのように金属イオンを放出するかを試験しました。最後に、酸化亜鉛ナノ粒子を充填したポリマー製被覆材は良好な抗菌作用を示しました。

研究成果の学術的意義や社会的意義
The obtained results are of both high practical and academic importance. They open up avenues to new-generation biodegradable biomedical materials incorporated with a range of nano-containers that can release various metal ions and drugs, which may significantly accelerate would healing.

研究成果の概要（英文）：Thin nanosheets of biodegradable polymer incorporated with nanoparticles were produced in this study. When in contact with skin, such biologically friendly polymer materials will release proper metal ions to promote wound healing.
Using laser ablation in liquid, we first produced nanoparticles containing ions of Zn, Fe, Cu and Mg. The nanoparticles were then incorporated into polymer nanosheets thus making a dressing material for wound healing. Then, we tested how such nanosheets release metal ions in contact with physiological solution. All incorporated nanoparticles were found to release their corresponding ions within a few hours after immersion, which is very important for practical use of wound dressings. Finally, polymer dressings loaded with ZnO nanoparticles demonstrated good antibacterial action. Thus, the developed materials show promise as next-generation wound dressings which not only release proper metal ions (to promote wound healing) but also suppress bacteria.

研究分野：Nanomaterials for biomedical use

キーワード：Laser ablation　Nanoparticles　Nanocontainers　Polymer nanosheets　Ion release　Wound dressing　Antibacterial behavior

## １．研究開始当初の背景

The treatment of burn wound is known to be a long-lasting and complex process that requires new therapeutic options to improve and accelerate the healing progress [1-3]. An ideal wound dressing should accelerate one or several stages of the healing processes including the inflammatory phase, the migratory phase, the proliferative phase, and the remodeling phase. A variety of wound dressings are available and still widely used to treat such wounds [1-3]. And even though the properties of ultimate wound dressings are well identified, major difficulties arise when it comes to combining them within the same material [1-3]. In other words, realizing an optimal combination of desired properties in one wrapping material is still challenging, and new materials with improved properties are highly anticipated. In addition, even though it is well known that metal ions like Zn, Mg, Fe and Cu are necessary for efficient wound healing [4], their incorporation into dressings used for wound treatment was not attempted yet.

## ２．研究の目的

Novel materials with advanced properties are anticipated for many bio-medical applications, including burn wound treatment. The purpose of this research project was to develop new-generation wound-wrapping biomedical materials based on bio-friendly thin nanosheets of PLLA (poly(L-lactic acid)) loaded with nano-containers / nanoparticles that can enhance wound healing through releasing appropriate metal ions. For this, the Research Team aimed at: (i) taking advantage of the laser ablation in liquid (LAL) as a technique to prepare all necessary nanostructures [5,6]; (ii) incorporating the nanostructures into thin nanosheets of bio-friendly polymers; (iii) study how the nanosheets release metal ions, and (iv) finally study how the nanosheets loaded with nano-containers suppress bacteria and promote wound healing processes.

The overall goal of this project was to develop new-generation wound-wrapping materials based on biodegradable polymer nanosheets (NSs) incorporated with nanoparticles (NPs) which serve as reservoirs of Zn, Mg, Cu and Fe ions. The latter metal ions will be released in contact with wound/skin and accelerate regeneration healing processes.

## ３．研究の方法

To achieve the above mentioned overall goal, we planed to (i) prepare different NPs by using the LAL approach, (ii) incorporate them into bio-friendly polymer NSs, and (iii) study how they release metal ions and affect bacteria that are potentially harmful for wound healing. Experiments *in vivo* were also planned in the future, in order to test how the prepared nanosheets promote wound healing on animals.

## ４．研究成果

### 4.1. Preparation of nanoparticles and their incorporation into polymer nanosheets.

As a first step, we prepared various nanoparticles (NPs) via laser ablation in liquid (LAL), applying two different lasers available and several liquid media (chloroform, water, ethanol, methanol). Figure 1 (left) presents a photograph of as-prepared colloid of $ZnCl_2$ NPs produced via ablating Zn plate immersed in chloroform. It is seen that a homogeneous colloid with Zn-containing NPs was prepared conveniently in chloroform, being immediately ready for further processing (embedment into polymer NSs). Similarly, colloids containing other metals (Fe, Cu, Mg, as oxides, metals, or chlorides) were also prepared by varying both laser parameters and liquid medium. When ablated in chloroform, the colloids were ready for direct use for mixing with PLLA polymer and spin-coating. Such colloids were found to have NPs mainly based on metal phase mixed with chloride and with some oxide phase as admixture. Whereas, when ablated in water or alcohols, the NPs had more oxide phase in their composition. However, prior to further processing, such NPs had to be centrifugated and re-dispersed in chloroform for further spin-coating as PLLA nanosheets. Interestingly, as will be shown below, no significant effect of phase composition on NP behavior as reservoirs of metal ions was observed in this work.

**Fig.1**: Cuvette with colloidal solution of $ZnCl_2$ nanoparticles prepared via laser ablation in liquid (left) and free-standing PLLA nanosheet incorporated with such nanoparticles (right), floating on water. The nanoshteet is about 3x3 $cm^2$ and about 50 nm thick, which provides superior flexibility and adhesion on any surface.

To produce larger quantities of $ZnCl_2$ NPs, a commercial $ZnCl_2$ salt was laser-irradiated in order to produce smaller NPs with sizes more appropriate for embedment into thin PLLA nanosheets. It was found that over time, laser irradiation of $ZnCl_2$ micropowder dispersed in chloroform led to a gradual decrease in particle size. Eventually, $ZnCl_2$ NPs prepared via laser irradiation were also incorporated into PLLA nanosheets.

As a second step, the laser-produced NPs were incorporated into PLLA nanosheets. This was achieved via spin-coating mixtures of PLLA polymer and colloidal NPs in chloroform as solvent. Figure 1 (right) shows a free-standing PLLA NS incorporated with $ZnCl_2$ NPs floating on water after peeling it off from the substrate. The thickness of the nanosheet was ~50nm, which makes it a very flexible structure with perfect adhesive properties. For comparison, various commercial products (NPs of metal oxides with well-defined particle sizes: CuO, ZnO, and MgO) were also used at this stage. This allowed us to vary and control the amounts of loaded metal ions more precisely, as well as to compare the behavior of different phases as containers with metal-ions incorporated into PLLA NSs.

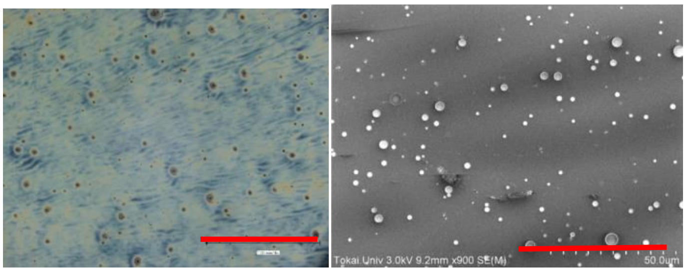


**Fig.2**: Optical image (left) and SEM image of two nanosheets incorporated with $ZnCl_2$ (left) and ZnO (right) NPs. Scale bars indicate 1 mm (left) and 50 micrometers (right).

We started from preparing PLLA nanosheets incorporated with one metal, after which NSs incorporated with 2 and 3 types of metal-containing NPs were also prepared. Upon preparation via spin-coating onto a sacrificial layer of PVA, the nanosheets were then easily peeled off as the sacrificial layer dissolved in water, leaving the nanosheets floating as free-standing films. Figure 2 shows optical microscopy (left) image and scanning electron microscopy (SEM) image (right) of two nanosheets incorporated with $ZnCl_2$ and ZnO NPs, respectively. It is well seen that incorporated NPs are uniformly distributed across the NSs, which is important for their efficient ion release when applied as dressing on and around wound location.

### 4.2. Ion release from prepared polymer nanosheets.

Figure 3 demonstrates how PLLA nanosheets embedded with different NPs released metal ions when immersed into physiological solution (0.9 % aqueous NaCl with pH 7.4). The solution simulated human body fluids and therefore was kept at +37 $^{o}$C. Aliquots were taken for ICP-MS analysis after certain periods of time. Figure 3 only presents results for several NSs, while similar trends were observed for all nanosheets incorporated with either one or 2-3 types of NPs (thus with 1 or 2-3 ions of different metals). Interestingly, as seen in Fig.3 (right), Zn-containing NPs with different composition (oxide and chloride) showed very similar dynamics of $Zn^{2+}$ ion release over time. It is clearly seen in Fig.3 that metal ions were actively released by nanosheets within 4, 10 and 6 h (for Cu, Fe and Zn, respectively), after which steady concentrations were reached.

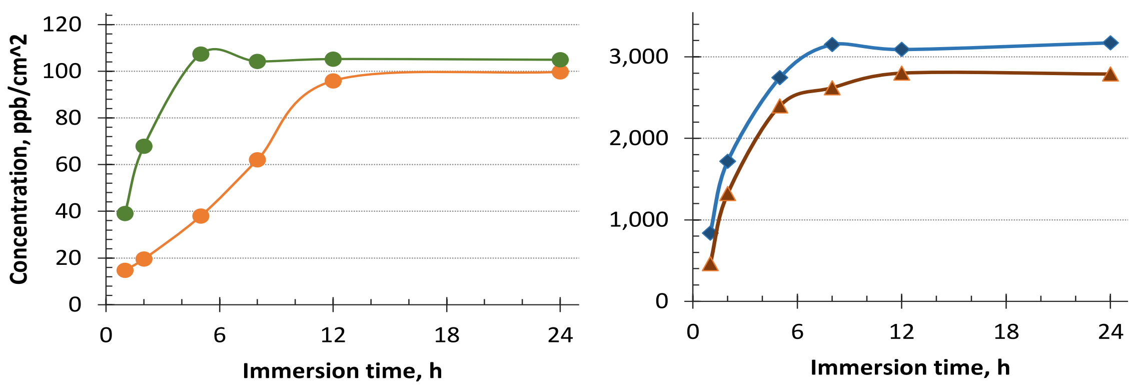


**Fig.3**: Ion release (in ppb/cm$^2$) over time from nanosheets incorporated with Cu (orange) and Fe (green) ion containing nanoparticles (left) and incorporated with Zn containing nanoparticles (right). The nanoparticles in right panels were $ZnCl_2$ (brown) and ZnO (blue).

The obtained results look very promising as dressing materials applied onto skin wounds are typically kept there for ~1 day and are expected to provide drugs or ions incorporated into them within a few hours upon their placement.

**4.3. Antibacterial performance of ZnO-loaded PLLA matrices.**

Along with promoting wound healing, ideally, biomedical materials being developed as wound dressings should also suppress pathogenic bacteria that are potentially available around the wound area. That is why we also tested the prepared PLLA materials loaded with laser-prepared ZnO NPs as biomaterials with potential antibacterial performance. For this, PLLA matrices electrospun as mats (see Fig.4) were then loaded with ZnO NPs prepared in water (left) and in air (right). Two strains of bacteria (*S. aureus* and *E. coli*) were tested (see Table 1 below).

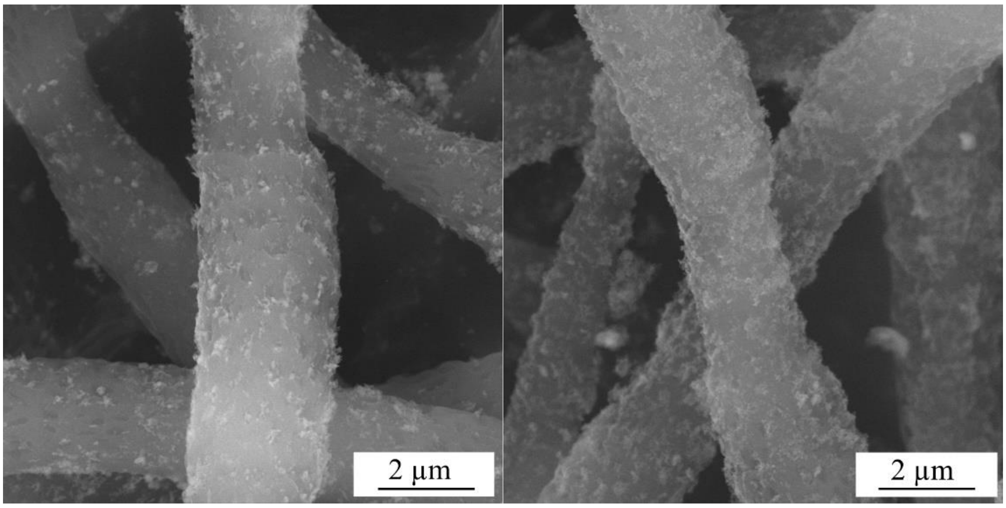


**Fig.4**: SEM images of model wound dressing tissues based on PLLA scaffold loaded with ZnO nanoparticles produced in water (left) and in air (right). Nanoparticles are seen as whitish powder atop the polymer fibers.

It was found that both ZnO nanomaterials, i.e. produced by laser in water and air, demonstrated good antibacterial activity towards *S. aureus*, with the activity of the NPs prepared in air being significantly higher (see Table 1 below). The activity of both dressings towards *E. coli* was comparable and less pronounced, whereas a good inhibiting effect was observed. Importantly, it should be noted that in case of wound dressings, it is the activity against *S. aureus* that is of much higher importance than that against *E. coli*. More details can be found in our publication on this matter (see ref. [7] below).

The results presented in Table 1 are very promising as they show that, in addition to promoting wound healing through releasing important metal ions (such as those of Zn, Mg, Fe and Cu), the developed PLLA dressings incorporated with ZnO NPs can also provide antibacterial effect and suppress malicious bacteria around wound area.

**Table 1**: Antibacterial activity of ZnO nanoparticles loaded onto PLLA matrix. Data are given for nanoparticles prepared in water (ZnO_water) and in air (ZnO_air). Strains of *S. aureus* and *E. coli* were tested.

| Sample | The Level of Growth | | Antibacterial Activity (A = F − G) |
|---|---|---|---|
| | Control $F = \lg C_t - \lg C_0$ | Sample $G = \lg T_t - \lg T_0$ | |
| *S. aureus* (+) | | | |
| ZnO_water_PLLA | +3.18 | −1.62 | +4.80 |
| ZnO_air_PLLA | | −2.48 | +5.66 |
| *E. coli* (−) | | | |
| ZnO_water_PLLA | +2.95 | +1.53 | +1.42 |
| ZnO_air_PLLA | | +1.67 | +1.28 |

**4.4. Gas sensing and photocatalytic performance of prepared nanomaterials.**

Apart from the above described experiments, some of metal-oxide nanomaterials prepared within the framework of this project were tested as potential gas –sensors. More specifically, our laser-generated ZnO NPs prepared by two different lasers in water, as well as $SnO_x$ nanomaterials prepared in water,

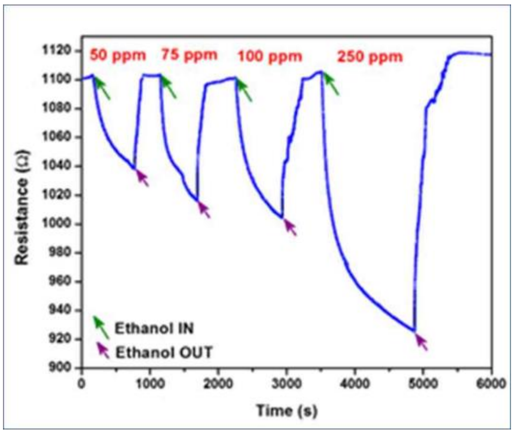


**Fig.5**: Dynamic-response curve of ZnO NPs prepared by ns- pulsed laser towards different concentrations of ethanol.

demonstrated sensing of ethanol (see Fig. 5 below), while ZnO-$SnO_x$ nanomaterials produced via LAL of Sn-Zn alloy exhibited sensing response towards ammonia. Importantly, all these results were obtained at room temperature, which implies the nanomaterials produced by lasers can be very promising for gas sensing to develop new-generation gas sensors working at room temperature. More details on gas sensors developed within the framework of this project can be found in works [8-10]. Finally, the ZnO nanomaterials produced by means of LAL in this project were also tested as photocatalytic materials decaying methylene blue in water medium. Their performance was compared, showing somewhat higher performance for ZnO NPs produced by nanosecond pulsed laser [11].

## References:

[1] Y. Okamura et al., **Adv. Mater**. 25, 545 (2013).
[2] J. Verbelen et al. **Burns** 40, 416 (2014).
[3] Y.H. Loo et al., **Biomaterials** 35, 4805 (2014).
[4] S.L. Kavalukas, A. Barbul, **Plast. Reconstr. Surg.** 127, 38S (2011).
[5] H.B. Zeng, X.W. Du, S.C. Singh, S.A. Kulinich, et al. **Adv. Fuct. Mater**. 22, 1333 (2012).
[6] K.Y. Niu, J. Yang, S.A. Kulinich et al., **J. Amer. Chem. Soc.** 132, 9814 (2010).
[7] E.A. Gavrilenko, D.A. Goncharova, I.N. Lapin, A.L. Nemoykina, V.A. Svetlichnyi, A.A. Aljulaih, N. Mintcheva, S.A. Kulinich, **Materials** 12, 186 (2019).
[8] T. Kondo, Y. Sato, M. Kinoshita, P. Shankar, N. Mintcheva, M. Honda, S. Iwamori, S.A. Kulinich, **Jpn. J. Appl. Phys.** 56, 080304 (2017).
[9] M. Honda, T. Kondo, T. Owashi, P. Shankar, Y. Ichikawa, S. Iwamori, S.A. Kulinich, ***New J. Chem.*** 41, 11308 (2017).
[10] N. Mintcheva, A.A. Aljulaih, S. Bito, M. Honda, T. Kondo, S. Iwamori, S.A. Kulinich, **J. Alloy. Compnd.** 747, 2018, 166 (2018).
[11] N. Mintcheva, A.A. Aljulaih, W. Wunderlich, S.A. Kulinich, S. Iwamori. **Materials**,11, 1127 (2018).

## ５．主な発表論文等

〔雑誌論文〕（計 7 件）

(1) E.A. Gavrilenko, D.A. Goncharova, I.N. Lapin, A.L. Nemoykina, V.A. Svetlichnyi, A.A. Aljulaih, N. Mintcheva, S.A. Kulinich, Comparative study of physicochemical and antibacterial properties of ZnO nanoparticles prepared by laser ablation of Zn target in water and air. ***Materials***, 査読有, vol.12, 2019, art.no. 186. [ DOI:10.3390/ma12010186 ]
(2) M. Kinoshita, S.A. Kulinich, K. Noda, S. Iwamori, Effect of surface morphology of ZnO layers deposited onto QCM on their sensing behavior during ethanol detection. ***Sensors & Materials***, 査読有, vol.30, 2018, pp.2773-2781. [ DOI: 10.18494/SAM.2018.1964 ]
(3) N. Mintcheva, A.A. Aljulaih, W. Wunderlich, S.A. Kulinich, S. Iwamori, Laser-ablated ZnO nanoparticles and their photocatalytic activity towards organic pollutants. ***Materials***, 査読有, vol.11, 2018, art. no 1127. [ DOI: 10.3390/ma11071127 ]
(4) N. Mintcheva, A.A. Aljulaih, S. Bito, M. Honda, T. Kondo, S. Iwamori, S.A. Kulinich, Nanomaterials produced by laser beam ablating Sn-Zn alloy in water. ***Journal of Alloys & Compounds***, 査読有, vol.747, 2018, pp.166-175. [ DOI: 10.1016/j.jallcom.2018.02.350 ]
(5) M. Honda, T. Kondo, T. Owashi, P. Shankar, Y. Ichikawa, S. Iwamori, S.A. Kulinich, Nanostructures prepared via laser ablation of tin in water. ***New Journal of Chemistry***, 査読有, vol.41, 2017, pp. 11308-11316. [ DOI: 10.1039/c7nj01634d ]
(6) T. Kondo, Y. Sato, M. Kinoshita, P. Shankar, N. Mintcheva, M. Honda, S. Iwamori, S.A. Kulinich, Room temperature ethanol sensor based on ZnO prepared via laser ablation in water. ***Japanese Journal of Applied Physics***, 査読有, vol.56, 2017, art. no. 080304. [ DOI: 10.7567/JJAP.56.080304 ]
(7) M. Honda, T. Goto, T. Owashi, A.G. Rozhin, S. Yamaguchi, T. Ito, S.A. Kulinich, ZnO nanorods prepared via ablation of Zn with millisecond laser in liquid media. ***Physical Chemistry Chemical Physics***, 査読有, vol.18, 2016, pp. 23628-23637. [ DOI: 10.1039/c6cp04556a ]

〔学会発表〕（計　**24**　件）

(1) A. Mussin, S. Iwamori, S.A. Kulinich, Synthesis of Fe-ion-containing nanoparticles via laser ablation and their embedment into polymer nanosheets for wound healing, レーザー学会学術講演会第39回年次大会; 2019.01.12- 2019.01.14, Tokyo.
(2) A. Mussin, S. Iwamori, S.A. Kulinich, Nanosheets of Degradable Polymers Embedded with Fe-ion-containing Nanoparticles as a Biomedical Material for Wound Healing; 第25回材料科学若手研究者討論会; 2018.09.05, Yokohama.
(3) M.Q.H. Ishak, S.A. Kulinich, Y. Okamura, S. Iwamori, Biodegradable polymer nanosheets incorporated with nano-containers as dressing for burn wound healing; International Nanotech & NanoScience Conference and Exhibition NanoTech France 2017; 2017.06.28-2017.07.01, Paris (France).
(4) M.Q. Hafzan Ishak, T. Kondo, Y. Okamura, S.A. Kulinich, S. Iwamori, Characterisstics of zinc-containing nanoparticles prepared via laser ablation in liquid and their application for biomedical polymer nanosheets, Korea-Japan International Symposium on Materials Science and Technology; 2016.11.15- 2016.11.18, Gyeongju, Korea.
(5) M.Q. Hafzan Ishak, T. Kondo, Y. Okamura, S.A. Kulinich, S. Iwamori, Ion release from Zn-incorporated polymer nanosheets for biomedicine; 77th Autumn Fall Meeting of the Japan Society of Applied Physics; 2016.09.12- 2016.09.17, Niigata.

図書〕（計　0　件）
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