Tunable grating based on stressed liquid crystal
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It is known that a typical grating can diffract an incident light into various-order beams. Gratings based on liquid crystal (LC) have attracted considerable interest owing to their high birefringence and sensitivity to an applied field, and possess considerable potential for application in displays, photonics and optical communications. In this paper, we report a new LC grating using the newly developed stressed liquid crystals (SLCs).

The concentration of polymer used in a SLC device is between that in polymer dispersed liquid crystals (PDLCs) and polymer stabilized liquid crystals (PSLCs). Typically, PDLCs and PSLCs contain polymer concentrations at ~30–50 wt%, and <10 wt%, respectively. These devices are operated based on the light scattering effect, and required to apply a high electric field. Yet, the SLC device can be operated not only applying a voltage, but shearing the substrate. Fast response and large phase retardation can be achieved using a SLC grating. Both the intensity and polarization of the diffracted beams can be controlled.

The SLC films were fabricated by homogeneously mixing a nematic liquid crystal LC (85 wt%) with a Norland optical adhesive NOA65 (15 wt%) (as a photo-polymerizable monomer). The LC employed was K15 (Merck), which has an ordinary refractive index of \(n_o = 1.5309\), and a birefringence of \(\Delta n = 0.1754\). Drops of homogeneously mixed K15-NOA65 compound were then sandwiched between two indium-tin-oxide (ITO)-coated glass slides (25 \(\mu m\)) to produce a sample. Before UV exposure, the cell was heated to \(T = 120^\circ C\), at which LCs are isotropic. Then, the cell maintained at 120 \(^\circ\) C, was exposed under an unpolarized UV light (365 nm) through a grating mask (spacing is 50 \(\mu m\)). Curing UV intensity was 12 mW/cm² and the exposure time was 25 minutes [Fig. 1(a)]. Figures 1(b)-left and 1(b)-right illustrate the randomly and uniformly aligned LC domains dispersed in the polymer network before and after the sample was sheared, respectively. During shearing, one substrate of the SLC device was fixed, and the other substrate was sheared into various lengths (Fig. 1(b)-right).

Notably, observations of SLC grating were performed under an optical polarized microscope (OPM). All
cells were measured by applying a He-Ne laser (632 nm) as the probe beam at room temperature. Shearing length ($L_{\text{shear}}$) of the SLC grating was controlled by a micro-screw (precision is 1 μm), and the shearing direction was along the grating vector.

![Fig. 2. Images of an SLC grating under a polarized optical microscope before and after shearing (50 μm); P, polarizer; A, analyzer.](image)

The images of an SLC grating under an OPM before and after shearing are presented in Fig. 2. The regions in a cell corresponding to the opaque and transparent ones of the photo-mask after UV exposure are enclosed by red- and green-dotted lines (Fig. 1), respectively. The UV exposed regions (green-dotted lines) formed a polymer-rich scattering conformation, and stayed at a scattering state after stressing due to the formed dense polymer network (Fig. 2). This result is reasonable since monomer concentration is getting lower in transparent regions than that in opaque regions during polymerization. This results in the fact that some monomers in the opaque regions diffuse towards adjacent transparent regions to equilibrate the monomer concentration across the sample, and form dense polymer networks in the transparent regions. Then, UV light was then scattered by the formed polymer networks in the transparent regions into the adjacent opaque regions where light polymer networks formed by side-scattered UV light. Due to the diffusion of monomers, a dark line formed in the center of the opaque regions, where LCs were rich and well aligned along the grating vector. Under a crossed-polarizer OPM, the region with well-aligned LCs appeared dark [Fig. 2(a)]. After stressing, the dark line widened [Fig. 2(b)], meaning that more LC-polymer composites were aligned toward the grating vector during stressing [Fig. 1(b)]. Figures 2(c) and 2(d) present the images of the same conformation under a parallel-polarizer OPM. The regions with well-aligned LCs became bright, indicating that the LC alignment in opaque regions can change by stressing [Fig. 1(b)], and such an alignment change after stressing can be used to control the phase difference of an incident beam through the SLC grating. Like a typical LC device, a SLC grating can be controlled also by applying a voltage. Therefore, a SLC grating can be modulated by shearing or by applying a voltage to tune diffracted beam intensity and polarization demonstrated below.

![Graph showing intensity vs. $L_{\text{shear}}$.](image)
Fig. 3. Diffraction patterns of an SLC grating observed under, (a) P//A, (b) P⊥A, before stress (upper) and after shearing (bottom) with a Lshear = 50 μm; P, polarizer; A, analyzer.

Figure 3 shows the diffraction patterns of an SLC grating before and after shearing (Lshear = 50 μm) under parallel-polarizer (Fig. 3(a)) and crossed-polarizer (Fig. 3(b)). The polarization of the probe-beam was parallel to the grating vector. The polarization of the first-order diffraction was measured. The measurement was performed with the polarizer axis parallel to the grating vector, and the analyzer axis was rotated with the polarizer. Figure 7 presents measurement results. Due to the phase variation in the opaque regions under stressing, the polarization of the first-order beam can be controlled with shearing the sample.

Fig. 4. Variation of the zero- and first-order diffraction intensities with shearing length Lshear under a cross-polarizer condition with the polarizer axis at an angle of ~45° relative to the grating vector.

Figure 4 plots the measured intensities of the zero- and first-order diffractions by shearing various lengths under the cross-polarizer condition with the polarizer axis at an angle of ~45° relative to the grating vector. Each of these two orders is seen to be modulated as the phase retardation varied by shearing lengths (Lshear).

Fig. 5. Diffraction patterns of an SLC grating sheared with a length Lshear of (a) 0 μm, (b) 30 μm, and (c) 60 μm under the application of various AC voltages. Measurements were performed under the cross-polarizer condition with the polarizer axis at an angle of ~45° relative to the grating vector.

Diffraction patterns of the SLC grating were then observed by applying AC voltages with the sample sheared a length. The measurement was also performed under a cross-polarizer condition with the polarizer axis making an angle of ~45° relative to the grating vector. The diffraction patterns of SLC grating devices under different shearing distances and applying voltages are shown in Fig. 5. Figure 6 presents the measured relations between the first-order diffraction efficiency and applied voltage under the parallel-polarizer condition as the sample was sheared from 0–60 μm. The diffraction efficiency (η) is defined as η = I_d / I_i, where I_i and I_d are intensities of the probing and diffraction beams. Similar to the results obtained with the sheared sample (Fig. 4), a SLC grating can also be modulated electrically. Notably, the modulation effect becomes obvious as shearing length increases.
Fig. 6. Variation of the first-order diffraction intensity with the application of AC voltages under a parallel-polarizer condition with the polarizer axis at an angle of $\sim 45^\circ$ relative to the grating vector.

Fig. 7. Variation of the first-order beam with sample under stress. Measurements are performed with the polarizer axis parallel to the grating vector, and rotating the analyzer axis.

In conclusion, this study presents a novel SLC grating that can be modulated by shearing a length or applying an AC voltage. Both the intensity and polarization of diffracted beams from a SLC grating can be tuned. The device capable of tuning the intensity and/or polarization of diffracted beams is highly demanded in various optical systems. Thus, SLC gratings have a good potential for practical applications.
Product design evaluation model of child car seat using gray relational analysis

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The purpose of this study aims to search for the comfort relations of child car seats by performing an experiment of physiological measures, and applying a scientific effective algorithm to build the product design evaluation model for designing optimal child car seats. The fundamental theory of the new product design evaluation (abbreviated as PDE) model is based on an artificial intelligent technique, which is the analysis method of gray relation. The gray relational analysis (abbreviated as GRA) is one kind of measuring method to analyze the relations among discrete arrays in the theory of gray system. It depends upon the geometric shape of serial curve to judge whether they are extensively related or not.

The new PDE model is developed through two stages. The first stage is to perform an ergonomic experiment to find out the relations between the comfort and the interface pressures on the child subjects. The second stage is to construct a PDE model by applying the method of gray relational analysis with programming languages.

The construction of the PDE model is based on the GRA of gray systemic theory. The model applies the GRA to analyze the pressure parameters of child car seats, and to make the relative evaluation of user’s comfort to each car seat. The program languages used to construct the new PDE model can be Visual Basic or C++. In this study, we adopted the Visual Basic. The building process is described as follows:

A. Build the relation table between seat variables and seat pressure parameters: Through seat pressure experiment and pressure data calculation, the pressure parameters of each sample can be obtained. The mean of each sample is further derived to construct a seat’s variables and seat’s pressure parameters relationship table (see Table 1) as the basis of the gray relational analysis.

Table 1. Relationships between seat’s variables and seat’s pressure parameters.

<table>
<thead>
<tr>
<th>Seat no.</th>
<th>Seat angle</th>
<th>Soft mat</th>
<th>BCP</th>
<th>BPP</th>
<th>BCA</th>
<th>CCP</th>
<th>CPP</th>
<th>CCA</th>
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</thead>
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<tr>
<td>No. 1</td>
<td>20°</td>
<td>Non</td>
<td>1.3861</td>
<td>0.1569</td>
<td>44.10</td>
<td>16.4009</td>
<td>0.4689</td>
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<td>No. 2</td>
<td>20°</td>
<td>KR</td>
<td>1.4746</td>
<td>0.0932</td>
<td>63.33</td>
<td>13.9042</td>
<td>0.1896</td>
<td>320.43</td>
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<tr>
<td>No. 3</td>
<td>20°</td>
<td>KQ</td>
<td>1.5094</td>
<td>0.1172</td>
<td>57.71</td>
<td>12.7967</td>
<td>0.2247</td>
<td>306.24</td>
</tr>
<tr>
<td>No.</td>
<td>Angle</td>
<td>Material</td>
<td>BCP</td>
<td>BPP</td>
<td>BCA</td>
<td>CCP</td>
<td>CPP</td>
<td>CCA</td>
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<tr>
<td>No. 4</td>
<td>20°</td>
<td>HD</td>
<td>1.8857</td>
<td>0.0961</td>
<td>50.71</td>
<td>13.8502</td>
<td>0.2066</td>
<td>299.19</td>
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<tr>
<td>No. 5</td>
<td>30°</td>
<td>Non</td>
<td>2.7709</td>
<td>0.1695</td>
<td>85.19</td>
<td>12.9149</td>
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<td>276.48</td>
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<td>No. 6</td>
<td>30°</td>
<td>KR</td>
<td>3.2473</td>
<td>0.1161</td>
<td>118.67</td>
<td>9.5665</td>
<td>0.1630</td>
<td>306.86</td>
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<tr>
<td>No. 7</td>
<td>30°</td>
<td>KQ</td>
<td>3.1535</td>
<td>0.1168</td>
<td>123.14</td>
<td>10.1308</td>
<td>0.1687</td>
<td>306.67</td>
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<tr>
<td>No. 8</td>
<td>30°</td>
<td>HD</td>
<td>2.3438</td>
<td>0.1071</td>
<td>99.24</td>
<td>9.4686</td>
<td>0.1709</td>
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<td>No. 9</td>
<td>40°</td>
<td>Non</td>
<td>2.9875</td>
<td>0.1917</td>
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<td>11.5937</td>
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<td>No. 10</td>
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<td>3.3883</td>
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<td>KQ</td>
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<td>No. 12</td>
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<td>3.1536</td>
<td>0.1342</td>
<td>115.43</td>
<td>7.9237</td>
<td>0.2141</td>
<td>277.24</td>
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</table>

Note: Back Contact Pressure = BCP; Back Peak Pressure = BPP; Back Contact Area = BCA; Cushion Contact Pressure = CCP; Cushion Peak Pressure = CPP and Cushion Contact Area = CCA

B. Proceed the gray relational analysis: Utilize the table 1 to process the gray relational analysis. Use Visual Basic to complete the evaluation model.

According to this evaluation model, product designer or related design develop personnel (the main model users) can proceed product design and development as well as product assessment and comparisons. It can provide an objectively scientific comfort-evaluating method to eliminate subjectively personal errors in the design process. In addition, it can effectively offer the design criteria of child’s comfort for car safety seats.

Results of the experiment and the gray relational evaluation can be summed up and discussed as the following:

1. From the Results of total gray relational evaluation, while arranging KR and HD soft mat into pairs and adjusting the angle of back-resting support at 30°, the child’s comfort level is the optimal.
2. A larger angle of seats can share the pressure load of buttocks, but if not adding soft mat, on the contrary, it will increase the peak value of back pressure and lead to reduce child’s comfortability. Thus, while desiring to increase angles of seat, the choice of soft mats should be emphasized in order to spread the peak values of pressures.
3. In the aspects of soft mat textures, there is an obvious difference between non-soft mat and other three soft mats. In other words, the total values and peak value of back and buttock pressures, as well as the utility value of contact area for seat without soft mat are less than those of other three soft mats. While proceeding with the total evaluations by applying GRA, the gray relational intensities of the three soft mats are obviously higher than those of non-soft mat. Thus, adding these three soft mats will obviously enhance the child’s comfortability.
4. In the aspects of comparisons of three soft mats, for the softest one, KQ with hardness 100.78 N, although the utility values of back and seat contact areas are higher, the total values and peak value of pressures are less than KR and HD soft mats, with hardness 116.46 N and 135.38 N, respectively. While performing the total evaluations in gray relational analysis, the KQ soft mat is not better than KR mat. The reason maybe is the same as the study of Sprigle and Chung who indicated that softer mats may cause becoming deformed too much and cannot spread contact pressures effectively. Thus, the child’s comfort will be lower due to larger contact pressures and higher peak value of pressures.
5. Results of the relations among pressure parameters show that the percentage of the peak value of seat’s back pressure occurred in the scapulas or the lumbar vertebrae is 81%, and 60% in the ischium tubercles or the thighs. Although the relations of experimental parameters and the occurred areas are not very sure. Results shown in Fig. 1 also indicate that the most frequently occurred areas of the peak value for seat’s back pressure are on the scapulas or the lumbar vertebrae while for seat’s pressure are on the ischium tubercles or the thighs. Therefore, in the design of child car seats, designers should design a profile chair, which fits child’s anthropometrical profiles to avoid producing peak values of back-supporting and buttock-supporting pressure.

![Fig. 1. The areas of peak values of back-support pressure and peak values of buttock-support pressure.](image)

To summarize the above results, we know that the choice of soft mats will affect the pressure parameters. Choosing an adequate soft mat can effectively reduce peak value of pressures and increase child’s comfortability. In addition, different angles of back-resting support can also affect pressure parameters. While increasing the angles of seats, designers should make use of soft mat design to reduce the peak value of pressures and simultaneously utilize profile chair or mat design to avoid producing new pressure loads. Results of this study by applying GRA are consistent with results of experimental statistics. Hence, the effect of this PDE model by applying GRA is right. This PDE model can also applied to different chair parameters, such as evaluating the relations between different chair’s contours and child’s comforts, the pressure affections of adding different accessories of chairs, and so on. Therefore, the construction of the evaluation system will be helpful to the design decisions.

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Selective Liquid-Phase Oxidation of InGaAs and Application to Metal–Oxide–Semiconductor InAlAs/InGaAs Metamorphic HEMT Without Gate Recess
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A selective, low cost, low temperature (30–70 °C), liquid phase oxidation of InGaAs using metal or photoresist as the mask has been proposed, and the oxide film composition and some process issues are also evaluated. Finally, the application of InAlAs/InGaAs MOS-MHEMT without gate recess has also been demonstrated. Without gate recessing, the gate oxide is obtained directly by oxidizing the InGaAs capping layer in a growth solution. As compared to its counterpart MHEMT, the MOS-MHEMT makes the proposed low-temperature and selective liquid-phase oxidation suitable for high power applications. Also, the InAlAs/InGaAs MOS-MHEMT samples exhibit the advantages of growing sidewall passivation and native gate oxide at the same time.

Recently, a simple and selective liquid-phase oxidation on GaAs-based materials operated at near-room-temperature (30–70°C) has been proposed and investigated. Based on this, various materials have been used to grow native oxides, such as Si, InP, AlGaAs, InGaP, InGaAs, InAlAs, and so on. Liquid-phase oxidation takes place by in-diffusion of oxygen at the oxide-semiconductor interface, such that a fresh interface region can be achieved due to the original semiconductor surface contaminants ending up on the oxide surface. With the technique, the growth of the native oxide film can be controlled well with good reproducibility at low temperatures. Additionally, using the electroless technique, neither vacuum nor gas condensation equipment nor assisting energy source is needed. Consequently, the liquid-phase oxidation not only exhibits the lowest process temperatures and system complexity but also provides oxides with a comparable and even superior quality.

The selective oxidation process is schematically illustrated in Fig. 1. First, the PR was coated on the In₀.₅₃Ga₀.₄₇As layer, the pattern of which was designed by the photolithographic processes. Then, the sample was transferred into the growth solution for oxidation. An oxide layer can be grown only on a bare InGaAs surface that is not covered by PR. After removing the PR using acetone, the final selectively oxidized structure can be obtained. The oxide film can also be etched using diluted HF solution, and the InGaAs seems to be consumed due to the loss of oxide species according to the scanning electron microscopy (SEM) image. As shown in Fig. 2, a high contract between InGaAs and oxidized InGaAs area on the top surface can
also be seen by the SEM image.

For In_{0.52}Al_{0.48}As / In_{0.53}Ga_{0.47}As MOS-MHEMT application, the MHEMT epitaxial structure was grown by metalorganic chemical vapor deposition on a semi-insulating GaAs substrate as shown in Fig. 3(a). The measured room-temperature Hall mobility and sheet carrier concentration were 7000 cm²/V s and 2 x 10^{12} cm⁻², respectively. The fabrication started with mesa isolation by wet etching down to the buffer layer. The ohmic contacts of the Au/Ge/Ni metal were deposited by evaporation and were then patterned by lift-off processes, followed by rapid thermal annealing (RTA). Then, applying the liquid-

phase oxidation procedure without gate recess, the wafer was directly immersed into the growth solution to generate an In_{0.53}Ga_{0.47}As gate oxide at 50°C for a period of time (e.g., 1 h). After this, the oxide films selectively and simultaneously selfaligned to passivate the surface and the sidewalls as shown in Fig. 3(b). Utilizing the liquid-phase oxidation, the proposed application used the Au/Ge/Ni metal as a mask for selective oxide growth on InGaAs. Finally, the gate metal Au was deposited. The gate dimension and the drain-to-source spacing are 0.65 x 200 μm² and 3 μm, respectively. For In_{0.53}Ga_{0.47}As material, the oxidation rate is about 10–15 nm/h in the growth solution, with initial pH = 5.0 at 50°C without any pH...
control, which is lower than that of In\textsubscript{0.15}Ga\textsubscript{0.85}As material (15–20 nm/h). The thickness of the as-grown oxide film is ~42 nm evaluated using an ellipsometer. A heterogeneous composition of the as-grown In\textsubscript{0.15}Ga\textsubscript{0.85}As oxide was found in the XPS depth profile. According to the XPS signals of In-3d, Ga-3d and As-3d core level indicate that the oxide films are composed of the compound of In\textsubscript{2}O\textsubscript{3}, Ga\textsubscript{2}O\textsubscript{3}, and As\textsubscript{2}O\textsubscript{3}.

Figures 4(a) and (b) show the drain current density $I_{\text{DS}}$ vs the drain-to-source voltage $V_{\text{DS}}$ of reference MHEMT and MOS-MHEMTs, respectively. Besides, good pinch-off and saturation characteristics are obtained. Due to the higher energy barriers at the gate interface, the MOS-MHEMT can be operated at higher $V_{\text{DS}}$ and gate-to-source voltage $V_{\text{GS}}$ than those of the counterpart MHEMT, which can enhance the current driving capability; this is promising for realizing high-power device applications. While the conventional Schottky-gate MHEMT may suffer from lower gate-swing voltage and lower breakdown voltage, which may limit the applications of the device.

For device fabrication, the photolithographic process is necessary; consequently, the as-grown oxide has the opportunity to expose to the developer directly. In order to study the etching phenomena of the oxide in chemical solutions such as the developer, experiments on immersing the oxide into chemical solutions for a certain period and measuring the variation of its thickness by the ellipsometer were performed. Figure 5 shows a plot for the thickness of the as-grown In\textsubscript{0.15}Ga\textsubscript{0.85}As oxide without PR as a function of etching time in the diluted D-35 developer (pH 12.80), the AZ 300MIF developer (pH 13.25), and the standard buffer solution (pH 10.0). The basis of the AZ 300MIF developer is tetramethylammonium hydroxide (TMAH), and the basis of the D-35 developer is NaOH. As shown in the figure, the oxide without PR starts to be etched in the diluted D-35 developer of pH 12.80 for 5 s.

In summary, Low-temperature (30–70 °C) selective liquid-phase oxidation of InGaAs using photoresist or
metal as the mask is proposed, and the oxide film composition is evaluated. Further, the application of the InAlAs/InGaAs metal-oxide-semiconductor metamorphic high-electron-mobility transistor (MOS-MHEMT) is also demonstrated. Without gate recessing, the gate oxide is obtained directly by oxidizing the InGaAs capping layer in a growth solution. In comparison, the InAlAs/InGaAs MOS-MHEMT is a good candidate for high-power applications.
Oct-3/4 expression reflects tumor progression and regulates motility of bladder cancer cells
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Cancer and embryonic stem cells exhibit similar behavior, including immortal, undifferentiated, and invasive activities. Here we demonstrate that in clinical samples bladder tumors with intense expression of stem cell marker Oct-3/4 (also known as POU5F1) are associated with further disease progression, greater metastasis, and shorter cancer-related survival compared to those with moderate and low expressions. We used RT-PCR and immunohistochemical approaches to examine Oct-3/4 expression in a panel of clinical bladder TCC samples illustrating a portion of RT-PCR results in which various levels of Oct-3/4 mRNA were detected in four human primary bladder carcinoma specimens, but not in normal human bladder tissue (Fig. 1A). Further immunohistochemical staining of frozen sections confirms Oct-3/4 expression in superficial bladder tumor tissues, but not in normal tissues (Fig. 1B). H&E-stained bladder tumor with moderate Oct-3/4 expression served as a comparison (Fig. 1B, lower right panel). Vast majority of Oct-3/4-positive tumor tissues suggest that Oct-3/4 expression in human primary bladder carcinomas might induce a cascade of gene expression to promote tumorigenesis.

We then systemically studied 57 patients with histologically confirmed superficial bladder TCC and 10 cases of non-neoplastic bladder tissues obtained by cystourethroscopic biopsies. Paraffin-embedded sections from tumor specimens were stained with H&E confirming the presence of at least 70% tumor cells. Overall, Oct-3/4 expression was seen in all tumor tissues but not in non-neoplastic bladder tissues. Of these tumor tissues, there were 25 (43.9%) with intense Oct-3/4 expression, 8 (14.0%) with moderate expression, and 24 (42.1%) with low expression. However, no significant association was found between Oct-3/4 expression and tumor grade, stage, morphology, and multiplicity. Univariate analysis revealed that multiplicity at diagnosis was the risk factor for tumor recurrence. Most importantly, tumors with intense Oct-3/4 expression were associated with further disease progression and greater metastasis compared with those with moderate or low expression (Fig. 1C). Furthermore, intense Oct-3/4 expression was also a risk factor for disease progression in bladder cancer subgroups, including grade II or III, as well as stage Ta-T1 or T2. Patients with low Oct-3/4 expression had longer cancer-related survival than those with high expression, as assessed by Kaplan-Meier survival analysis and log-rank test (Fig. 1C). The poor patient survival rate in Oct-3/4 high expression group may be attributed to greater disease progression and higher metastasis. Therefore, intensity of Oct-3/4 expression provides the prognostic information for patients with superficial bladder TCC. RT-PCR and immunoblot studies also demonstrate Oct-3/4 expression in human and murine bladder cancer cell lines, as well as murine HM-1 ES cells that served as the positive control for Oct-3/4 expression (Fig. 1D). In contrast, Oct-3/4 expression was not detectable in normal murine NMuMG epithelial cells. Of note, immortalized human epithelial (SV-HUC-1) and murine fibroblast (NIH3T3) cells expressed much smaller amounts of Oct-3/4 compared with bladder cancer cell lines. We also confirmed immunohistochemically Oct-3/4
expression in MBT-2 and HM-1 cells, but not in NIH3T3 cells. Taken together, these results implicate Oct-3/4 as a novel tumor biological and prognostic marker for bladder cancer.

A, examination of Oct-3/4 expression in four human bladder tumor tissues (PT1~PT4) and one normal bladder tissue (NT) by RT-PCR. B, immunohistochemical detection of Oct-3/4 expression in three representative tumor sections (intense, moderate, and low) of human superficial bladder TCC, but not in normal bladder tissue (200× magnification). The upper right panel shows the magnified image (320× magnification) of the area indicated by the box in the upper middle panel, which reveals intranuclear staining of Oct-3/4 in tumor tissues. An Oct-3/4 moderate-stained section was also subjected to H&E-staining (lower right panel). Bars, 100 μm. C, correlation between Oct-3/4 expression and tumor progression (top left), metastasis (top right), and cancer-related survival (bottom left). D, detection of Oct-3/4 expression in bladder cancer cell lines as well as murine ES cells, as determined by RT-PCR and immunoblot analysis. Note that either none or very small amount of Oct-3/4 was detected in normal epithelial cells (SV-HUC-1 and NMuMG) and fibroblasts (NIH3T3). Expression of β-actin served as the loading control.

Oct-3/4 overexpression enhanced migration and invasion of bladder cancer cells in vitro, we then
investigated in animal models whether the metastatic potential of bladder cancer cells correlated with Oct-3/4 expression. In the animal model of experimental pulmonary metastasis in which cancer cells were injected into mice via the tail vein, tumor nodules in the lung was detected more frequently in those inoculated with MBT-2/Oct-3/4 cells compared to those injected with the control cells, as revealed by gross examination and H&E staining (Fig. 2A). In particular, there was a significant increase in pulmonary metastatic colonization, both in lung weight as well as in the number and size of the tumor nodules (Fig. 2B). In the spontaneous metastatic model in which tumor cells were inoculated subcutaneously into the mice, the number of visible metastatic nodules in the lung was greater in mice inoculated with MBT-2/Oct-3/4 cells than in those receiving control cells (Fig. 2C). Histological sections also revealed that mice inoculated with MBT-2/Oct-3/4 had more tumor lesions (Fig. 2D). Taken together, these results indicate that Oct-3/4 overexpression promoted the metastatic behavior of bladder cancer cells in vivo.

FIGURE 2. Overexpression of Oct-3/4 promotes metastasis in animal models of experimental pulmonary and spontaneous metastases. A and B, in the mouse model of experimental pulmonary metastasis, C3H/HeN mice (n = 8) were inoculated with MBT-2/Oct-3/4 or control cells via the tail vein and killed 30 days after tumor cell inoculation. A, gross appearance of two representative lungs from each group of mice (left). The length of the small square corresponds to 1 cm. Representative H&E-
staining of the lungs of two mice from each group (right). The scale bars shown on 40× images correspond to 1 mm. Note that the numbers and sizes of tumor nodules indicated by arrows were increased in mice inoculated with MBT-2/Oct-3/4 cells. B, wet lung weight of each group of mice (left). Number of tumor nodules on the surface of the lung from each group of mice (right). columns, mean of eight determinations; bars, SEM. C and D, in the model of spontaneous metastasis, C3H/HeN mice (n = 8) were inoculated s.c. with MBT-2/Oct-3/4 or control cells and killed 60 days after tumor cell inoculation. C, number of metastatic nodules on the surface of the lung from each group of mice. The mean values are indicated by horizontal bar. D, representative lung histology stained with H&E of each group of mice. The arrows denote tumor nodules, and the scale bars shown on 40× and 100× images correspond to 500 μm.

The expression of Oct-3/4 in bladder cancer promoted tumor progression and metastasis, which may accounted for by the activation of MMP-13, MMP-2, and MMP-9 expressions by Oct-3/4. Therefore, the expression of Oct-3/4 may contribute to the group of bladder cancer patients with poorer survival, and Oct-3/4 may act as a novel target for cancer therapy especially in cancers with high propensities for metastasis. In the syngeneic MBT-2 tumor model in immunocompetent mice, we also conclude that Ad5WS4, an oncolytic adenovirus driven by the Oct-3/4 promoter, may serve as a possible therapeutic strategy for bladder cancer with greater effectiveness and cancer-specific potentials.