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Injectable liquid alkali alloy based-tumor thermal ablation therapy

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ORIGINAL ARTICLE

Injectable liquid alkali alloy based-tumor thermal ablation therapy

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Abstrac

The alkali metal was recently found to be a very useful agent for inducing minimally invasive tumor hyperthermia therapy. However, the solid-like metal makes it somewhat inconvenient to perform the surgery. Here, to overcome this drawback, the NaK alloy in liquid state at room temperature was proposed as a highly efficient thermal ablative agent for tumor treatment. For illustration purposes, the functionalized liquid NaK alloy at a mass ratio 1:1 was obtained and an amount of 0.35ml was injected into *in vitro* pork. The sizes of the damage region and temperature response were measured. It was found that significant temperature increase by a magnitude of $>80^\circ$C can easily be obtained. This produced a large coagulation and necrotic area within selected areas for *in vitro* tests and the necrotic region volume is three times that of the NaK injection quantity. Furthermore, for the *in vivo* experiment, breast EMT6 tumor in mouse was subjected to treatment by NaK alloy. Tumor was harvested after the experiment to assess its viability. Histological section showed complete necrosis at the target site. These conceptual results demonstrate that using injectable liquid alkali alloy to ablate tumor is rather promising. This study also raised interesting issues waiting for clarification in future technical and animal studies aiming to assess efficacy, side effects and safety of the new therapy.

Key words: *Tumor hyperthermia, alkali metal, injectable liquid alloy, thermal ablation, minimally invasive therapy*

Introduction

In 2000, ten million people developed a malignant tumor and a total of 6.2 million died from this disease. According to the world cancer report, the cancer occurrence rates could further increase by 50% to 15 million new cases in the year 2020 (1). Clearly, cancer treatment has become a major global concern especially at the present day.

Traditional surgical resection of solid tumor is only valid for well-defined and primary tumors situated near non-vital organs and recommended mainly for those patients with pre-treatment midline shift whenever possible (2). Unfortunately, only a small portion of the patients are eligible for such treatment. In addition, invasive characteristic and high morbidity of such surgery seriously limit its radical application.

As an alternative, thermal ablation therapies are being widely tried in the treatment of tumor as a minimally invasive method. Up to now, various thermal ablation strategies have been successfully invented. The most typical heat generation principles generally include high intensity focused ultrasound (3), radiofrequency (4), microwave (5), and laser-induced thermal therapy (6) etc.

The key for a thermal ablation technique is to generate a clear boundary in a desired time, and to prevent surrounding normal tissue from being burned. High intensity focused ultrasound can cause localized hyperthermia at a predictable depth extracorporeally. However, absorption of the ultrasound energy by the bone may lead to normal tissue burn (7). MRI-guided focused ultrasound therapy provides a temperature monitoring system which would lead to a much better clinical effectiveness (8). Microwave is more suitable for the treatment of superficial tumors due to smaller penetration depth (5). RF can heat deep tumors, but needs local water cooling for safety of subcutaneous fat which is prone to overheating and pain (9). In a laser-induced thermal therapy, the high power density at optical fiber tips easily leads to carbonization of surrounding

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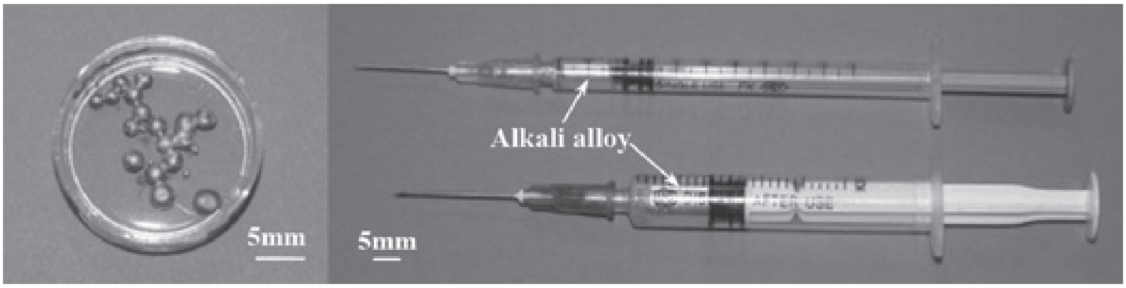

Figure 1. Optical image of (a) liquid sodium-potassium alloy sealed in kerosene at room temperature and (b) two types of sodium-potassium alloy injector.

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tissue (10). Improved probes designed with a saline-cooled application system can avoid carbonization at proper perfusion rate, laser energy and ablation time. If laser energy becomes higher than a certain threshold, it would result in carbonization of the Teflon sheath and the tissue even if applied under an increased saline perfusion rate or shorter exposure time (11). Thus, for larger tumors multiple probes were often needed simultaneously. As a consequence, trauma is generally inevitable.

Recently, we proposed a new conceptual strategy of utilizing solid alkali metal as the heat generation seeds to release a highly localized and powerful heating to kill tumor (12,13). Unlike most of the conventional heat delivery methods, this thermal ablation therapy requires no expensive and complex equipment. This is because reaction between an extremely small amount of alkali metal and intrinsically existing water in the biological body would produce significant enough temperature increase by a magnitude of >40°C at the target site. However, a potential limitation of this method lies in that the solid-form alkali metal, though in small size, appears not very convenient for injection and thus appears a little difficult for surgical operation.

Here, aiming to realize a highly flexible and almost noninvasive tumor treatment, we proposed for the first time to utilize liquid sodium-potassium (NaK) alloy for thermal ablation. NaK is usually stored in kerosene, which gives it a silvery metallic appearance (Figure 1 a). Compared with the pure alkali metal such as Na or K, the NaK alloy has a rather low melting point like −12.8°C when potassium mass content is 77.8% and is changeable according to its species concentration. It could stay at liquid state at room temperature in the concentration range of 40–90%. For clinical purposes, the liquid alloy can be sealed in a commonly used syringe. At the current stage, we delivered liquid NaK directly into the targeted tissue by a syringe needle with a diameter of 0.5 mm. The mechanical trauma due to insertion of the medical syringe is so small that can be ignored. In this sense, such surgery can be regarded as noninvasive since it does not cause any evident mechanical trauma afterwards. Besides, the performance appears much superior to most state-of-the-art percutaneous minimally invasive surgeries in which the typical diameters of the inserted probes generally range from 1.2 mm to 6 mm or even larger (14,15).

For the present operation, the liquid NaK is loaded in advance at room temperature in a common syringe (Figure 1 b). After being injected into the target tissue, a significant temperature increase or even combustion (Figure 2) can be observed, which immediately results in a powerful thermal ablation effect at only the tumor site. This is owing to the strong exothermic reaction between the alkali metal and the intrinsically existing water contained in the target tissues. Reaction between the sodium, potassium and water can be quantified by the following equations:

$$\mathrm{Na} + \mathrm{H_2O} = \mathrm{NaOH} + \frac{1}{2}\mathrm{H_2}\uparrow + 140.886\mathrm{KJ/mol} \quad (1)$$

$$\mathrm{K} + \mathrm{H_2O} = \mathrm{KOH} + \frac{1}{2}\mathrm{H_2}\uparrow + 140.008\mathrm{KJ/mol} \quad (2)$$

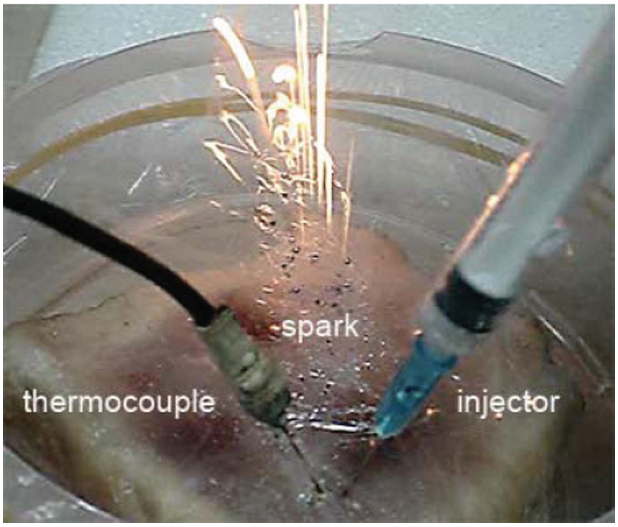

Figure 2. Combustion phenomena when NaK was injected into pork without protection gas.

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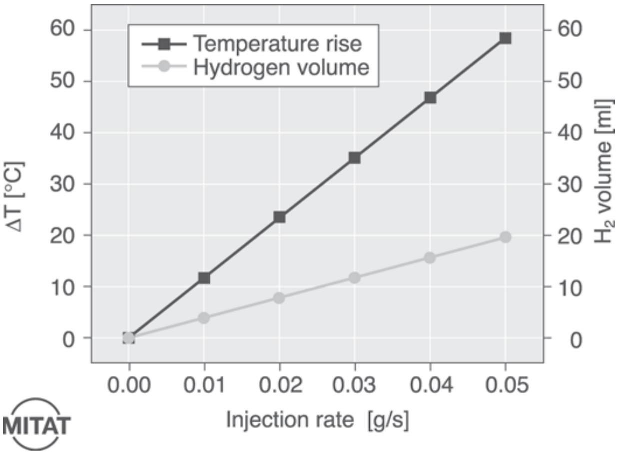

Figure 3. Theoretical calculation results of temperature rise and hydrogen volume as a function of injection rate. Here, heat release during reaction between NaK (mass rate 1:1) and water was calculated, under the condition that heat loss was ignored.

When NaK (mass ratio 1:1) was injected into 1 ml water at a certain rate, relationships between rejection rate and water temperature rise (heat loss was ignored for simplicity) and hydrogen volume can be calculated as shown in Figure 3. It demonstrates that the heat generation rate achieved is tremendously high. As to the hydrogen production, a hollow pipe can be placed at the reaction site to guide the gas out to the external environment.

High heat release by the NaK allows them to serve directly as an anticancer therapeutic agent. One obvious merit of the present method still lies in that the remaining reactants such as Na^+ and K^+ are friendly absorbable by the living tissues, since they consist of the most basic element of the physiological fluid. Meanwhile, the remaining weak alkali environment would do good to prevent surviving tumor cells from regeneration. In addition, the significantly dissolved OH^{-1} will not cause adverse effects for the biological body. In the following, experiments will be carried out on injection of liquid NaK *in vitro* and an intratumoral *in vivo* case for a noninvasive lethal heating of malignant cells.

Material and methods

Functionalized NaK

Olive oil was chosen as alloy-protective agent due to its high compatibility with biological body. Sodium and potassium with the same quantity are placed in olive oil under nitrogen at room temperature. The alloy is thus made by stirring in a glass jar. The melting point for NaK alloy at 1:1 rate is about 10°C (16). So it always stays at liquid state at room temperature. The alloy appears relatively stable in the protective agent. Then the alloy was treated by a 20 min exposure to ultraviolet irradiation to remove any bacteria. Before treatment, liquid metal was absorbed and sealed in a syringe as shown in Figure 1 b. The syringe needle is 0.5mm in diameter, 18 mm in length, just as commonly used in clinics.

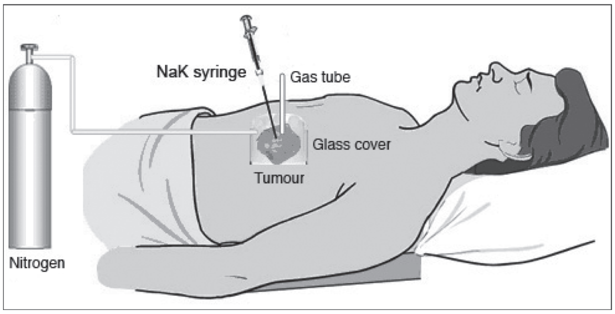

Figure 4. Schematic map for administering the tumor treatment via injectable alkali alloy. Transparent glass hood was fixed at target tissue for observation and prevented alkali alloy from splashing. Nitrogen gas was introduced into the glass shield via a pipe connected to a pressure nitrogen bottle. An exhaust pipe is connected with the glass hood.

Temperature measurement

The temperature sensors used in the *in vitro* and *in vivo* experiments were the T-type copper-constantan thermocouples which were connected to an Agilent 34970 multimeter, USA. To prevent the chemical corrosion of the reactant, a 0.35mm diameter copper–constantan thermocouple was chosen. The thermocouple was calibrated in advance and an accuracy of ±0.1°C was obtained. During the entire period of injecting NaK and the subsequent 2 min after stopping injection, the temperature was continuously recorded once every second.

Establishment of security protection system

As sketched in Figure 4, a safeguard system is essential to reduce the risk of the experiments. A transparent glass hood was fixed at the target tissue for an easy observation and to prevent alkali alloy from splashing. Nitrogen was introduced into the glass shield via a pipe connected to a pressure nitrogen bottle with a flow rate controllable by a valve. When the valve was switched on, nitrogen would generate positive pressure in the glass hood which can help release reaction gas like hydrogen through a pipe.

In vitro measurement to evaluate thermal effect of NaK

An *in vitro* study on porcine muscle was performed. A porcine tissue was placed in a glass shield. After

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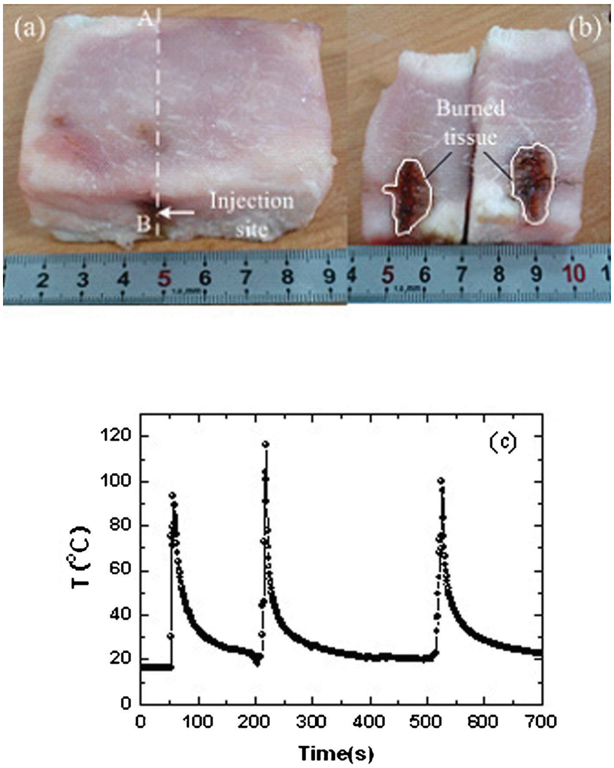

Figure 5. The outputs of the experiment on *in vitro* pork tissue, in which 0.1ml, 0.15ml and 0.1ml sodium and potassium alloy was injected into the inner of the tissue, respectively. (a) Illustration of the positions from where the pork tissue was sliced after the experiment. (b) Profiles of the burned area, in which the pork tissue was sliced off via the white line AB. The shape of the burned tissue looks like a water drop. (c) The temperature response of tissue during reaction process in which 0.1ml, 0.15ml and 0.1ml NaK was added into the pork at the same location.

the glass hood was filled with nitrogen, 0.1 ml, 0.15 ml and 0.1 ml NaK was added into the tissue at the same place. Temperature response near the injection site of tissue during the reaction process was recorded.

Tumor-bearing animal model

An animal experiment was performed in compliance with the International Laboratory Animals Care Convention. EMT6 tumor cells were grown in RPMI (Roswell Park Memorial Institute) 1640 medium, the cells were harvested, washed, and resuspended in sterile phosphate buffer solution. 0.2 ml of the EMT6 tumor solution was injected into the oxter of female BALB/c mice (6~8 weeks). When tumors in the oxter of the donor mice reached its greatest dimension like 1 cm to 1.5 cm, the tumors were harvested under sterile conditions and cut into 1 mm cubes. After grinding and filtering, their tumor cells were resuspended. 0.2 ml of suspension was injected into each mouse. Ten days later, the tested mouse was anesthetized by intraperitoneal injection of 0.2 ml pentobarbital sodium (concentration 6.7 mg/ml), the resulting EMT6 tumor was injected directly with NaK. After the experiment, the mouse was euthanized. Its tumor was harvested and evaluated to gather evidence of the thermal injury. Tumor was cut into 5μm frozen sections and prepared for histological assessment with hematoxylin and eosin staining.

Results

NaK alloy heating of in vitro tissue

The thermal ablation appearance and temperature outputs for the *in vitro* tests on porcine tissue are depicted in Figure 5, in which 0.1 ml, 0.15 ml and 0.1 ml NaK was added into the pork at the same place, respectively. Figure 5 a and b show injection point and the profile of the burned area, in which a necrosis area was specifically indicated using a white curve. Figure 5 c presents the temperature response near the injection site of tissue during the reaction process. With each injection, a strong temperature pulse by a magnitude of about 80°C was realized at the injection site.

NaK alloy induced cytotoxicity of malignant breast tumors in vivo

A BALB/c mouse bearing EMT6 tumor size at about 1.5 cm in greatest dimension underwent a direct introtumoral injection of NaK alloy. After treatment, the mouse was killed. Histopathology sections from tumor injected with NaK revealed complete thermal necrosis of the tumor tissue with a longitudinal 10 mm and transversal 4 mm zone of thermal injury (Figure 6 a). In contrast, tumors around the injection site had no evidence of tumor cell death in the histologic specimens. It can be seen from section (Figure 6 a) that tissue can be divided into three types: Normal zone, transition zone and destroyed zone, respectively. If using the corresponding N, T, D point to represent normal, transition and damaged zones, respectively, as marked in (a), each section can be magnified by 200 times under an optical microscope as depicted in Figure 6 b, c and d, respectively. Clearly, as shown in Figure 6 b, cells in the normal zone are intact. At the transition zone connecting normal tissue and necrosis tissue, the top left is not destroyed in Figure 6 c and cells at bottom right are necrotic. Cell structure in the damage zone appears to have completely disappeared and its nucleus is dissolved, as shown in Figure 6 d.

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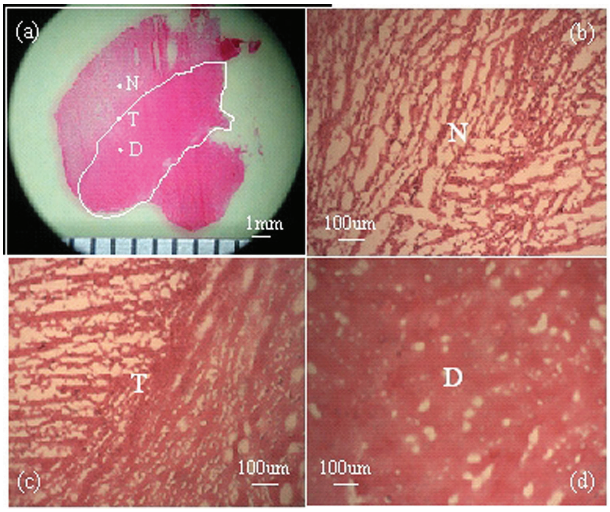

Figure 6. (a) Hematoxylin/eosin staining of EMT6 tumor after in vivo treatment shows the tissue structure damage within the area occupied by NaK alloy. (b) Region near N point demonstrates integrate cell structure, original magnification, 200. (c) Region near T point demonstrates the top left is not destroyed and cells at bottom right were necrotic, original magnification, 200. (d) Region near D point demonstrates that the cell structure appears to have completely disappeared and its nucleus was dissolved, original magnification, 200.

Discussion

Localized high intensity thermal destruction of carcinoma cells has been demonstrated in an *in vitro* test. The cylindrical regions of liquefied necrosis are seen in Figure 5 a and b. The diameter of the cylinder is about 8 mm in average, and the height of the cylinder is about 20 mm. The volume of the cylinder is thus estimated as 1cm^3, which is three times the NaK injection quantity. Longitudinal of the melting region is consistent well with length of injector needle. The diameter of the melting region is larger than that of the needle, confirming that tissue necrosis was mainly induced along radial direction. Relationship between NaK injection quantity and tumor volume can be expressed as:

$$V = \gamma \pi r^2 h \qquad (3)$$

where V is injection quantity, ml. r is radius of damaged areas, cm. h is length of damaged areas, cm. γ is NaK melting effectiveness of tissue. Here, γ is about 0.33.

In addition, *in vitro* temperature analysis revealed that NaK treated tissue resulted in an average temperature increase of 80°C at the injection quantity of 0.1 ml. This therapy raised temperature well above the damage threshold necessary to induce irreversible tissue damage (17). The temperature increases reported here were acquired at a depth of $\approx$1.8 mm beneath the surface near the injection needle.

Further examination of thermal response also demonstrated the powerful heating effect to tumor in in vivo tumor tissue. Overall, these findings correlated well with gross histology, in which different structures were observed in NaK treated tumor in the regions where thermocouples data suggested there should be irreversible tissue damage. There is an obvious boundary between damaged areas and undamaged areas. Histology also demonstrated loss of cell microstructure, dissolution of nucleus (Figure 6 d), which identified common markers of thermal damage.

Compared with conventional thermal ablation techniques such as laser, microwave, and radiofrequency hyperthermia etc., the injectable NaK alloy-induced exothermic reaction with tissue allows to obtain a much higher energy density. In addition, tremendous heat can be released at only the target tissue via an extremely small amount of NaK. This characteristic overcomes the high cost of previous heating techniques. What is more, the target region surrounding the NaK injection site becomes strongly alkalotic (see Eq.(1) and (2)). Clearly, such alkaline environment would play an important role in denaturing protein, collapsing cell structure and enhancing thermosensitivity of tumor cell.

From a clinical perspective, security for prevention of risk during operating NaK ablation is critical. Therefore, standard approval procedures and a comprehensive protection system should be established in the near future.

Due to the fact that NaK reaction with tissue is a calefactive process, temperature is an important index to monitor the damage zone. Infrared radiation is proportional to the temperature distribution and related with the degree of blood perfusion of tissue. Considering that infrared thermography is a noninvasive method to monitor the temperature distribution, it can be adopted for administrating the ablation process. As an alternative, a three-dimensional mapping of temperature changes can also be possible with magnetic resonance (MR) and is related to the relaxation time T(1), the diffusion coefficient (D), or proton resonance frequency (PRF) of tissue water. The use of temperature-sensitive contrast agents and proton spectroscopic imaging can provide absolute temperature measurements. Therefore, MRI can also be adopted for treatment planning and monitoring of the temperature distribution during thermal ablation in the near future (18).

Conclusion

Through introducing liquid NaK alloy, we have achieved localized, irreversible thermochemical

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ablation of tissue both *in vitro* and *in vivo*. In the former case, the tissue due to injection of NaK was destroyed with a damage area of three times the injection quantity. Histological section ensured a successful irreversible thermal destruction. Initial results demonstrated the potential utility of NaK as a thermal ablation agent to treat malignant tumors.

Finally, we should also emphasize that the new therapy is still at its incubation stage. Quite a few efforts are critically needed on either animal or clinical tests to exactly access size, efficacy and side effects of the present method. In addition, a safeguard system for clinical practice needs to be improved. The package of NaK and safety regulation requests further standardization. The present research is expected to serve as a valuable guidance for future endeavour along this direction.

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