Microneedle-based delivery devices for cancer therapy: a review


Pll: S1043-6618(19)31632-9
DOI: https://doi.org/10.1016/j.phrs.2019.104438
Reference: YPHRS 104438
To appear in: Pharmacological Research

Received Date: 7 August 2019
Revised Date: 28 August 2019
Accepted Date: 29 August 2019

Please cite this article as: Moreira AF, Rodrigues CF, Jacinto TA, Miguel SP, Costa EC, Correia IJ, Microneedle-based delivery devices for cancer therapy: a review, Pharmacological Research (2019), doi: https://doi.org/10.1016/j.phrs.2019.104438

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2019 Published by Elsevier.
Microneedle-based delivery devices for cancer therapy: a review


a CICS-UBI – Health Sciences Research Centre, Universidade da Beira Interior, Av. Infante D. Henrique, 6200-506 Covilhã, Portugal.

b CIEPQF – Departamento de Engenharia Química, Universidade de Coimbra, Rua 13 Silvio Lima, 3030-790 Coimbra, Portugal.* Corresponding author. Tel.: +351 275 329 002; Fax: +351 275 329 099; e-mail: icorreia@ubi.pt.

Graphical abstract

Abstract:

Macroscale delivery systems that can be locally implanted on the tumor tissue as well as avoid all the complications associated to the systemic delivery of therapeutics have captured researchers’ attention, in recent years. Particularly, the microneedle-based devices can be used to efficiently deliver both small and macro-molecules, like chemotherapeutics, proteins, and genetic material, along with nanoparticle-based
anticancer therapies. Such capacity prompted the application of microneedle devices for the development of new anticancer vaccines that can permeate the tumor tissue and simultaneously improve the effectiveness of therapeutic agents.

Based on the promising results demonstrated by the microneedle systems in the local administration of anticancer therapeutics, this review summarizes the different microneedle formulations developed up to now aimed for application on cancer therapy (mphasizing those produced with polymers). Additionally, the microneedles’ general properties, type of therapeutic approach and its main advantages are also highlighted.

**Keywords:** Microneedles; Cancer; Vaccines; Gene delivery; Chemotherapy.

1. **Introduction**

Despite all the efforts, cancer remains as one of the leading health problems affecting the world population. In fact, recent data clearly show that the cancer incidence has been increasing in the last decades [1, 2]. On the other hand, both the conventional (e.g. chemotherapy and radiotherapy) and novel (e.g. nanomedicines) anticancer therapeutics present sub-optimal efficacies [3-5]. Further, the clinical translation of highly promising nanoparticle-based anticancer therapies has been severely hindered by their low capacity to accumulate within the tumor tissue (i.e. less than 0.7% of the administered dose reach the tumor site) [6, 7]. Moreover, the nanomedicines’ high complexity also difficult the synthesis procedures scale-up and the reproducibility of the therapeutics outcome [5]. Therefore, in the recent years, researchers started to re-explore macroscale delivery devices (e.g. microneedle systems and hydrogels) that can mediate a local and controlled delivery of the therapeutic agents at the tumor region [8-10].
Particularly, microneedle devices have been showing promising results that support its application as anti-tumoral therapies. The concept of microneedle-based drug delivery systems was firstly described at more than three decades ago and their practical clinical application started to be highly pursued within the past 15 years [11]. Since then, microneedles have been fabricated using a myriad of materials including metals, polymers, glass, and ceramics as well as in a wide variety of shapes and arrangements, in order to meet the desired clinical requirements [12-14]. Further, during the last decade, extensive preclinical evaluation studies have been performed by the scientific community for validating the microneedles’ application as drug delivery systems in the biomedical field. To date, microneedles have been used in the preclinical and clinical investigation to i) enhance the passive transdermal delivery, ii) evaluate the vaccines’ antigenicity, iii) evaluate altered protein pharmacokinetics and pharmacodynamics, iv) determine pain and other perceptions associated with microneedle delivery and lately v) transport anticancer therapeutics [15-20].

In cancer therapy, the application of microneedle devices has been explored to trigger anticancer immunologic responses (e.g. antigens, immune adjuvants, genetic material) or to deliver anticancer compounds (e.g. drugs and nanoparticles) [21-25]. In that way, the local application of microneedle-based systems can increase the distribution and the amount of drug that reaches the deeper regions of the tumors, while minimizes the therapeutics leakage to adjacent tissues and the side effects [18, 26, 27]. Further, the microneedles also facilitate the combination of different drugs in one single therapy as well as allows their temporally controlled release, which can be used in the drug development phase for screening the best therapeutic combinations and in the clinic to enhance the anti-tumoral effect [28-30]. Therefore, this review provides an overview of the different microneedle formulations described in the literature for cancer therapy, emphasizing those produced with polymers. The microneedles general properties, type of therapeutic approaches, and their main advantages are also highlighted in the following sections.
2. General Properties of Microneedles

Microneedle devices are composed of micron-size needles that can be organized in single structures or arranged in small arrays to mediate the localized delivery of therapeutic molecules [31, 32]. These micro-projections generally present dimensions around 50 to 250 μm in wide and lengths that can range from few micrometers to those as long as 1500 μm [32, 33]. In general, the microneedles application aims to create a transport pathway for the delivery of therapeutic molecules, bypassing the external barriers that limit the therapeutics penetration in the target tissue. Further, the microneedles devices are compatible with the delivery of both small and macromolecular therapeutics such as small drugs (e.g. doxorubicin), proteins (e.g. ovalbumin), genetic materials (e.g. pDNA and siRNA), or even nanomedicines [33-35]. Additionally, the microneedles are highly versatile and are regarded as less painful, damaging, and safer to use, when compared to conventional needles [13]. Moreover, the microneedles can be produced using several different materials (e.g. stainless steel, titanium, glass, and polymers) and fabrication methods [36-39]. Such results in a wide variety of microneedle designs that in general are categorized as solid microneedles, drug-coated microneedles, dissolving microneedles, and hollow microneedles [13, 31, 40]. As shown in Figure 1, these microneedle designs enable the delivery of therapeutics by different mechanisms, which can be explored for the temporally control of the therapeutics action.
Figure 1 – Representation of the main microneedle designs explored for drug delivery applications, (A) Solid microneedles, (B) Layered microneedles, (C) Dissolving microneedles, and (D) Microneedles drug reservoir.

Solid microneedles are usually applied for tissue pretreatment in order to increase the therapeutic agents’ delivery to the target tissue [41, 42]. In this approach, the microneedles insertion and subsequent removal originates micron-scale pores in the tissue, allowing the posterior application of a drug formulation, in the form of a drug solution or hydrogels, that will diffuse through the pores to deeper regions of the tissue [41, 43]. On the other hand, the microneedles can present a layered organization where the base layer confers mechanical support for insertion in the target tissue and the remaining layers contain the therapeutic molecules [44, 45]. In this way, after the microneedles’ application, the dissolution of the different microneedle layers will allow the diffusion of the therapeutic molecules to the tissue [46, 47]. Alternatively, the microneedles can present the therapeutic agent homogeneously dispersed in the microneedles’ polymeric matrix [48]. Therefore, the release of the encapsulated payload will occur in a process mediated by the microneedles’ degradation (e.g. polymer enzymatic breakdown, hydrolysis, or dissolution) [49-51]. Further, microneedles can be engineered to contain a drug deposit and hollow tips with empty channels that allow the passage of the drug solution to the target tissue [13, 52]. In this way, the selection and optimization of the microneedles design and materials allow the
controlled and localized delivery of therapeutic molecules directly to the target tissue and the ability to easily explore different drug formulations for achieving an enhanced therapeutic effect. In the following sections, the application of polymer-based microneedles aimed for treating cancer will be described, highlighting the microneedle device production method, therapeutic approach, and efficacy.

3. Microneedles anticancer vaccines

Cancer vaccination (immune and gene-based therapies) has been receiving a great attention by the scientific community in the last years, since it has been revealing promising anticancer results [53, 54]. In this field, the utilization of microneedles can be advantageous due to its capacity to overcome the skin’s stratum corneum layer and deliver its payload upon contact with the interstitial fluid, usually at a depth of superior to 200 µm [55, 56]. In fact, when compared to the conventional hypodermic needles, the microneedle-based delivery systems avoid the pain and discomfort of the hypodermic needle insertion in the skin as well as the creation of hazardous wastes [31, 34]. Further, the microneedles contain the vaccine (immune or gene-based) in a dried solid form, which increases its thermostability and facilitates the application on the target area [35, 57].

3.1. Immune therapies

Cancer immune-based vaccinations purpose to trigger a systemic immune response by the host in order to eradicate the tumor tissue (Figure 2) [58]. Such approaches usually explore the vaccine delivery to the skin, the largest immunological organ of the body, which is densely populated by antigen-presenting cells (APCs), i.e. macrophages, Langerhans, and dendritic cells [59]. These APCs, when activated, can stimulate the T (CD4⁺ and CD8⁺) and B cells and consequently induce a systemic antitumoral immune response [60, 61].
Figure 2 – Representation of the microneedles’ application as anticancer vaccines. The loading of peptides, monoclonal antibodies, immune adjuvants, or even genetic material on the microneedle devices aims to induce the activation of the immune cells triggering an antitumoral response by the host.

For that purpose, researchers have been exploring the application of microneedle patches, containing immunostimulatory adjuvants and/or antigens, as anticancer therapeutic approaches. Zaric et al. described the application of methylvinylether and maleic anhydride microneedles enriched with ovalbumin-loaded poly-
\textsubscript{D,L}-lactide-co-glycolide (PLGA) nanoparticles for triggering the antigen-specific immune response against ovalbumin expressing B16 melanoma tumors [62]. In this approach, the authors prepared ovalbumin loaded PLGA nanoparticles via water-in-oil double emulsion technique and then added the nanoparticles to a solution of methylvinylether and maleic anhydride that was poured on silicone templates to create the microneedles (19 by 19 array). These authors observed, in the \textit{ex vivo} assays, that the microneedles could penetrate the murine skin, reaching the dermal layer and the local dendritic cells. Further, they also observed that the transfected dendritic cells could migrate to the proximal lymph nodes and trigger the activation of CD8\textsuperscript{+} T cells and the production of
cytokines such as IFN-γ. Moreover, this immune system activation, mediated by the nanoparticles release from the microneedles, prevented the development of ovalbumin expressing B16 melanoma tumors for 13 days. In a similar way, Kim and colleagues developed a microneedle patch composed of Pluronic F127 and polyethylene glycol for the simultaneous delivery of resiquimod (R848) and tumor antigens [63]. The R848 is a ligand of the human toll-like receptor 7 and 8 (TLR 7/8) expressed by immune cells, like macrophages, dendritic and B cells. The R848 interaction with TLR 7/8 can trigger the production of IL-12, IFN-γ, and TNF-α and therefore stimulate the antigen-specific humoral and Th1 immune responses [64, 65]. Further, this device also mediated the simultaneous delivery of ovalbumin to direct the immune response towards E.G7-OVA (lymphoma cells expressing ovalbumin) tumor-xenografts. The microneedle array with 49 pyramid-shaped needles was prepared by depositing a mixture of Pluronic F127/R848 and PEG/ovalbumin and drying it at room temperature under vacuum in a polydimethylsiloxane (PDMS) mold. Then, it was also noticed that the microneedles could penetrate the superficial layers of the mouse skin and allow the diffusion of its payload to the adjacent cells. Moreover, the authors as well reported that upon dissolution, the microneedles form nanomicelles that could mediate the delivery of R848 and ovalbumin to RAW264.7 cells and, consequently, stimulate the production of cytokines as well as induce the macrophages maturation. On the other hand, the in vivo assays showed that the microneedles loaded with R848 and ovalbumin could not only activate the skin APCs but also migrate to lymph nodes. In fact, when comparing to the mice treated using hypodermic syringes, the authors reported that the microneedles could mediate the generation of higher levels of antigen-specific antibodies (37x increase for hypodermic needles and 46 x increase for microneedles) and cytotoxic T cells. This improvement in the immune response hindered the E.G7-OVA tumor growth (tumor weight: ≈2.5g in control group and ≈0.4g on the microneedles treated group). Additionally, extended necrotic areas were noticed in the tumor tissues treated with the microneedles (Figure 3).
Figure 3 – Evaluation of the ovalbumin and R848 loaded microneedles antitumoral efficacy on E.G7-OVA tumor bearing mice. (A) Timescale of the cancer cells inoculation and therapeutic vaccinations. (B) Analysis of the tumor size progression for 28 days. (C) Tumor weight measurements at day 28. Reprinted with permission from ACS Nano, Vol. 12 (10), Kim, et al., Enhanced Cancer Vaccination by In Situ Nanomicelle-Generating Dissolving Microneedles, 9702-9713. Copyright (2018) American Chemical Society.

On the other side, microneedles can be engineered to deliver antibody based immune therapies aimed to revert/by-pass the tumor cells’ immune suppressive signals. Ye and coworkers produced a hyaluronic acid-based microneedle array for mediating the delivery of anti-PD1 antibody (aPD1) and 1-methyl-DL-tryptophan (1-MT) to B16F10 melanoma tumors [66]. The aPD1 targets the PD-1 receptors expressed by T cells and therefore can avoid the cancer cells inhibitory signaling that prevent the T cells activation [67]. On the other hand, 1-MT is an inhibitor of the immunosuppressive enzyme indoleamine 2,3-dioxygenase favoring the occurrence of an enhanced immune
The conical shaped microneedle arrays (15 by 15) were produced using silicone molds. For that purpose, the authors chemically grafted the 1-MT to the hyaluronic acid chains, which subsequently were used to create micelles loaded with the aPD1. Then, the micelles were deposited on the silicone molds, dried, coated with additional 1-MT modified hyaluronic acid, and dried at room temperature under vacuum to obtain the microneedles. The authors observed that the 1-MT and aPD1 release occurred in response to the HAase-triggered degradation of the microneedles and micelles [66]. Further, the microneedles presented a fracture force of 0.41 N, which allowed them to penetrate the mice’s dorsum skin and, consequently, deliver its payload at a depth of 200 µm. Additionally, the microneedles administration on mice bearing B16F10 melanoma cells increased the retention of 1-MT and aPD1 at the tumor tissue. In fact, the accumulation of 1-MT on melanoma was 3-fold higher at day 1 and 5-fold higher at day 2 and 3 on the group treated with the microneedles than in that treated with free 1-MT. Moreover, the simultaneous delivery of aPD1 and 1-MT by the microneedles impaired the tumor progression (tumor area inferior to 50 mm², whereas in the control group the area was superior to 300 mm²) and increased the mice survival rate. On the other hand, Wang and colleagues explored the development of hyaluronic acid microneedles for the delivery of aPD1 and glucose oxidase loaded dextran nanoparticles for the treatment of skin cancer [69]. In this approach, the aPD1 and glucose oxidase loaded dextran nanoparticles were deposited on silicone molds that were subsequently filled with acrylate modified hyaluronic acid and N,N-methylenebisacrylamine allowing the microneedles formation (15 by 15 array) upon exposition to UV light. The glucose oxidase can mediate the generation of gluconic acid from the glucose present in the medium and consequently promote the nanoparticles dissociation and the aPD1 release [70]. The authors showed that the produced microneedles presented a failure force of 0.38N/needle, which allowed its penetration into the mouse skin up to 200 µm in depth. Additionally, in vivo assays where B16F10 mouse model of melanoma was used revealed that the administration
of aPD1 could efficiently stimulate the CD 8+ T cells infiltration into the tumor tissue (% of CD 8+ T cells increased from 30% to 43% when comparing the groups treated with free aPD1 and microneedles). Such improvement resulted in a significant tumor inhibition/regression as well as an increase in the mice survival rate, 40% of mice survived for 40 days after microneedles treatment, whereas the mice treated with free aPD1 only survived for 30 days.

3.2. Gene therapies

Apart from the application of microneedles for mediating the immune system activation through the delivery of antigens, adjuvants, or even antibodies, these macroscale delivery devices have been also explored for the administration of antitumoral gene therapies. Ali and colleagues developed a polyvinylpyrrolidone microneedle patch loaded with E6/E7 pDNA RALA nanoparticles for the treatment of cervical cancer [71]. The microneedles were produced using a micromolding process, by depositing a PVP solution containing E6/E7 pDNA/RALA nanoparticles on a negative mold. Additionally, the authors observed that upon manual application 90% of needle length could be inserted on the porcine skin and that after 15 min the microneedles tips were already completely dissolved within the skin layers. Further, the authors demonstrated that after microneedles administration the concentration of antibodies was 2 times higher than that of the control and the T cells were more responsive to HPV-16 oncogenic antigen expressing cells (TC-1) (IFN-γ levels in control ≈250 pg/mL and ≈530 pg/mL for microneedle immunized group). This enhanced immune response prevented the establishment of cervical tumors on 4 of the 9 mice treated with microneedles. Moreover, the authors also reported that the microneedles administration on mice bearing cervical tumors induced the regression of the tumor area, 246 mm² microneedle treated mice and 503.13 mm² for mice vaccinated with RALA-E6/E7 nanoparticles. Alternatively, Pan and coworkers utilized dextran/hyaluronic acid/polyvinylpyrrolidone microneedle patches for the delivery of
polyethyleneimine/STAT3 siRNA complexes to skin melanoma tumors (Figure 4) [72].

STAT3 is a protein overexpressed in several tumors (e.g. melanoma, breast, and prostate) that can prompt the cancer cells proliferation, angiogenesis, survival, and immune evasion [73]. These microneedles were produced by blending polyethyleneimine/STAT3 siRNA complexes with a dextran/hyaluronic acid/polyvinylpyrrolidone solution, followed by the casting in PDMS molds of pyramidal-shaped needles (12 by 12 arrays).

Figure 4 – Morphological characterization of the microneedle devices loaded with polyethyleneimine/STAT3 siRNA complexes. (A) Macroscopic photographs of the microneedles. (B) Scanning electron microscope images of the microneedle patches. (C) Analysis of the complexes loading on the microneedles through confocal laser scanning microscope.

The authors reported that almost 100% of microneedles tips penetrate the rat skin when forces superior to 20N are applied, microneedles have failure force 86N, which allows the delivery of the polyethyleneimine/STAT3 siRNA complexes at a depth of 330µm [73]. Further, the authors also observed that the topical administration of STAT3 siRNA mediated by the microneedles could reduce the STAT3 mRNA expression in 30% as well as induce the necrosis of 40% of the tumor cells. Such effect inhibited the growth of the melanoma tumor, registering a tumor weight 5 times lower than the control.

Table 1 - Overview of the microneedles devices as vaccines for cancer therapy.

<table>
<thead>
<tr>
<th>Microneedle Material</th>
<th>Production Method</th>
<th>Therapeutic agent</th>
<th>Needles Size</th>
<th>Model</th>
<th>Type of study</th>
<th>Results</th>
<th>ref.</th>
</tr>
</thead>
</table>

[116x103]
4. Local administration of anticancer therapeutic agents

In recent years, the microneedles arisen as an alternative to the chemotherapeutics systemic administration (Figure 5). This local administration of the therapeutic agents decreases the non-specific interactions with healthy tissues and consequently the side-
effects [4]. Further, when comparing to the intra-tumoral administration of liquid drug formulations, the microneedles provide a better spatial and temporal control over the therapeutics release to the tumor tissue, which can limit the drug leakage to the adjacent healthy tissues [76].

Figure 5 – Representation of the microneedles’ application as delivery devices for cancer therapy. The microneedles can transport and mediate the delivery of conventional anticancer drugs, photothermal and photodynamic agents as well as nanomedicines.

Zhao and colleagues developed an hyaluronic acid based microneedle patch loaded with 5-aminolevulinic acid for the photodynamic therapy of subcutaneous tumors [77]. The 5-Aminolevulinic acid can be converted into protoporphyrin IX (PPIX) in cell mitochondria, acting as a photosensitizer by mediating the production of highly reactive oxygen species upon light irradiation. The microneedles were produced by depositing a hyaluronic acid solution containing 5-aminolevulinic acid on PDMS molds of pyramidal-shaped tips (5 by 5 arrays). The authors observed that the microneedles could penetrate the rat skin up to 218±58 µm of depth and the drug content could reach the
600 µm in 2 min. Further, the authors also demonstrated that the photodynamic therapeutic effect mediated by the microneedles could induce a decrease on the tumor volume. In fact, mice treated with microneedles and subjected to the light irradiation (635 nm, 450 mW, for 10 min) presented a reduction of the initial volume of the tumor to 44% in just 7 days, whereas the group treated with free 5-aminolevulinic acid and light the tumor volume increased in 455% (Figure 6). Such data demonstrated that the microneedles-mediated local administration can improve the drug bioavailability, avoiding the non-specific interactions and degradation during blood circulation.

Figure 6 – Evaluation of the 5-aminolevulinic acid (ALA) loaded hyaluronic acid-based microneedle patches antitumoral efficacy. (A) Analysis of the tumor growth and (B) photographs of the tumor bearing mice. (C) Analysis of the tumor weight after 14 days and (D) photographs of the excised tumors. Reprinted from Journal of Controlled Release, vol. 286, Zhao, Xiao, et al., Tip-loaded fast-dissolving microneedle patches.
for photodynamic therapy of subcutaneous tumor, 201-209. Copyright (2018), with permission from Elsevier.

On the other hand, microneedles can also mediate the simultaneous delivery of different therapeutic agents such as drug-drug and drug-nanoparticle combinations for improving the efficacy of anticancer treatments. Bhatnagar and coworkers developed a polyvinylpyrrolidone/polyvinyl alcohol microneedle patch for the combinatorial delivery of doxorubicin and docetaxel to breast cancer tumors [29]. Doxorubicin and docetaxel are two chemotherapeutics commonly used to treat this type of cancer. Doxorubicin is a non-selective class I anthracycline that can induce the cell death by intercalating with the DNA and suppressing the macromolecules biosynthesis [78]. On the other hand, docetaxel interferes with the normal function of microtubule growth affecting the cytoskeleton flexibility and cell mitosis [79]. In this approach, the drugs were incorporated on the microneedles tips by dissolving them on the polyvinylpyrrolidone solution [79]. Then, this solution was deposited on a PDMS mold of pyramidal-shaped needles and coated with a polyvinylpyrrolidone/polyvinyl alcohol mixture to further increase the patch mechanical strength. The authors observed that this methodology allowed the production of microneedles with a compression strength superior to 5N, which endured the insertion on the mice skin being detected the drug delivery until a depth of 140µm. Further, the authors described that the microneedles patch was completely dissolved in 33 min, with 90% of the docetaxel being released in the first 15 min. Additionally, the in vivo studies performed on 4T1 breast tumors bearing mice revealed that the combinatorial delivery of doxorubicin and docetaxel was more efficient than the single treatment approaches leading to an impaired tumor growth. Moreover, when compared to the free drug administration, the increased mice survival rate and the long-term maintenance of the mice weight demonstrated that the microneedles-mediated chemotherapeutic administration also mitigate the drugs non-specific toxicity. In a similar approach, Pei et al applied polyvinylpyrrolidone...
microneedles for mediating the delivery of doxorubicin and indocyanine green to the synergistic chemo- and photothermal-therapy of osteosarcoma tumors [80].

Indocyanine green is a dye that can induce cancer cells death through the production of heat and/or singlet oxygen upon irradiation with near-infrared (NIR) light [81]. For this purpose, the authors encapsulated the indocyanine green on mesoporous silica nanoparticles and blended them with a solution, composed of doxorubicin and polyvinylpyrrolidone, that was casted on PDMS molds of pyramidal needles (10 by 10 arrays) [81]. The microneedle patches presented a compressive strength of 0.37N and achieved a complete penetration of skin stratum corneum when a force of 0.03N was applied. Moreover, the authors observed in vitro that upon NIR light irradiation (808 nm, 0.34 W.cm\(^{-2}\), 1 min), the microneedles could mediate an increase in the temperature to values superior to 50\(^\circ\)C. In fact, the microneedles application on subcutaneous tumors and subsequent NIR light irradiation (808 nm, 0.34 W.cm\(^{-2}\), 2 min) induced an increase in the temperature of the tumor tissue up to 48\(^\circ\)C. Additionally, the authors also reported that the combination of the photothermal effect with the doxorubicin action provoked a decrease in the tumor volume to 50\% and induced the necrosis of ≈80\% of the tumor cells. Hao and colleagues also explored the combinatorial application of nanoparticles and drugs, i.e. gold nanorods and doxorubicin, for the chemo- and photothermal-therapy of human epidermoid cancer therapy [82]. The gold nanorods are one type of NIR responsive nanomaterials, due to the surface plasmon resonance phenomenon, that can be used for mediating a photothermal effect [83]. In this strategy, the gold nanorods, doxorubicin, and hyaluronic acid were blended and casted on a PDMS template of 20 by 20 microneedle patches [82]. The authors demonstrated that upon NIR light irradiation (808 nm, 1 W.cm\(^{-2}\), 5 min), these patches could induce an increase in the temperature up to 65\(^\circ\)C. Additionally, this temperature increase could also expedite the doxorubicin release to the adjacent tissue. In the in vitro assays, the authors observed that the microneedles (loaded with the drug) were able to induce a similar cytotoxic effect to that attained through free drug administration.
Nevertheless, in the in vivo assays, the authors demonstrated that the microneedles local administration and controlled release of both gold nanorods and doxorubicin renders a superior antitumoral efficacy. In fact, the combinatorial treatment mediated by the microneedles resulted in the complete remission of the A431 tumors, contrasting with the slightly inhibited tumor growth on the stand-alone therapies (i.e. free drug, microneedles mediated chemotherapy, or microneedles mediated photothermal effect).

Similarly, Dong and coworkers developed a gold-nanocage and doxorubicin loaded hyaluronic acid microneedles for the transdermal photothermal and chemo-therapy of skin tumors [84]. For this purpose, PDMS molds were filled with a solution containing doxorubicin and gold nanocages. Then, the samples were dried, and a solution of hyaluronic acid was added to the previous PDMS molds, originating microneedles patches (10 by 10 arrays) with pyramidal needles enriched with doxorubicin and gold nanocages. The authors observed that the inclusion of the gold nanocages improved the microneedles mechanical strength, the young modulus increased from 68.9 to 224.9 MPa, which is crucial for facilitating the microneedles penetration of the skin’s stratum corneum and accomplish a 220 µm penetration depth. Further, these authors also demonstrated that the local administration of the microneedles on subcutaneous melanoma tumors could induce a temperature increase up to 55ºC when irradiated with a NIR light (808nm, 1 W.cm⁻², 1 min). This localized delivery of doxorubicin and gold nanocages to melanoma tumors peaked at 24h and the authors observed that the therapeutics still remained in the tumor tissue after 72h. Moreover, despite the authors did not report any significant difference on the antitumoral effect mediated by the microneedles and the intra-tumoral administration of free doxorubicin and gold nanocages (both treated tumors presented a size 83% smaller that the non-treated group), the microneedles successfully reduced the therapeutics non-specific toxicity, being observed a reduction on the mice weight in the free therapeutics treated group (Figure 7).
Figure 7 – Evaluation of the \textit{in vivo} antitumoral capacity of gold nanocages and doxorubicin loaded hyaluronic acid microneedles. (A) Analysis of the tumor volume progression during the 12 days. (B) Tumor weight measurements at day 12. (C) Body weight analysis and (D) Survival rates of the C57 mice. Reprinted with permission from ACS Applied Materials and Interfaces, Vol. 10 (11), Dong, et al., Au Nanocage-Strengthened Dissolving Microneedles for Chemo-Photothermal Combined Therapy of Superficial Skin Tumors, 9247-9256. Copyright (2018) American Chemical Society.

Alternatively, some authors also have been exploring the application of microneedle devices for the high-throughput screening of the tumors’ drug sensitivity. These approaches aim to simultaneously test a range of therapeutics on a \textit{in vivo} tumor as well as evaluate the patient individual responsiveness to a specific therapeutic agent or therapeutic combinations. For this purpose, Jonas and colleagues developed a microscale needle containing several drug reservoirs, each with a unique single agent or drug combination, that can be implanted inside the tumor for the short-term
evaluation of the tumor drug sensitivity (Figure 7) [30]. This cylindrical device with 820 µm of diameter and 4 mm in length, presented up to 16 drug reservoirs in its surface and could be implanted into the tumors through a biopsy needle. Then, the drugs can diffuse into different tumor regions of 200-300 µm, the drug diffusion can be controlled (i.e. delay or favor) by modulating the reservoir size, incorporation of polymer matrixes, and utilization of expansive hydrogels.

Figure 8 – (A) Representation of the microneedle device application as a drug screening device. The device can be implanted directly on the tumor tissue, allowing the drugs diffusion from the reservoirs to confined regions in the tumor. This behavior allows to evaluate the tumor-specific response to a determined drug. (B) Effect of the different reservoir characteristics on the drug release profile. The drug release kinetics
can be controlled by the reservoir size, drug-polymer matrix formulation, or by the utilization of expansive hydrogels. From Science Translational Medicine, Vol. 7, Jonas, et al., An implantable microdevice to perform high-throughput in vivo drug sensitivity testing in tumors, 284ra57. Reprinted with permission from AAAS.

The authors observed that this device could restrict the drug release from each reservoir to individual tumor regions and the therapeutics combination could be achieved by encapsulating the different compounds into the same reservoir. Further, the authors also reported that this control over the therapeutics release could match the intra-tumoral drug concentration achieved by the systemic administration of the therapeutics, i.e. tumor zones with drug levels of 8 to 13 mg/kg or 3 to 7 mg/kg, which strengthens the translation of the results obtained with this device to the clinic.

Moreover, the authors evaluated the device capacity to act as a predictor of the systemic efficacy in three human cancer mouse models (A375 melanoma, BT474 breast, and PC-3 prostate) demonstrating that the doxorubicin could induce the highest apoptotic index (55%) on A375 cells followed by BT474 and PC-3 cells (18% and 8%, respectively). These data were coherent with those obtained from the mice treated through intravenous administration, apoptotic index of 34.9% and 8.7% for A375 and PC-3 cells respectively. In this way, the authors also demonstrated that this microneedle device can be used to determine the most effective drug formulation towards a specific tumor. For example, the authors reported that for the microneedle device treated groups the paclitaxel presented the highest apoptotic index (54%) against triple-negative breast cancer tumors, followed by doxorubicin (36%), cisplatin (25%), gemcitabine (12%), and lapatinib (4%). Moreover, the microneedle device also allowed the authors to determine the best drug combinations, for example on BT474 tumors, the combination of doxorubicin with sunitinib leads to an increase of the apoptotic response from ≈3% to ≈5.5%, this apoptotic response was further improved when the doxorubicin was combined with lapatinib (13.45%). In a subsequent study,
Davidson and colleagues also utilized the previous microneedle device to demonstrate that pancreatic cancer cells can use extracellular proteins (e.g. albumin) as a source of amino acids, which can translate to a preferential uptake of albumin based nanocarriers and improve the tumor delivery of therapeutics [85].

Table 2 – Overview of the microneedles’ application as drug delivery devices for cancer therapy. N.A. – non applicable.

<table>
<thead>
<tr>
<th>Microneedle Material</th>
<th>Production Method</th>
<th>Therapeutic agent</th>
<th>Needles Size</th>
<th>Model</th>
<th>Type of study</th>
<th>Results</th>
<th>ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvinyl alcohol and polyvinyl pyrrolidone</td>
<td>Micromoulding 6x6 arrays</td>
<td>Doxorubicin and docetaxel</td>
<td>Height: 597.2 ± 31.5 μm; Base width: 245.8 ± 16.1 μm</td>
<td>4T1 tumor-bearing athymic nude mice</td>
<td>In vivo</td>
<td>Increased mice survival and impaired tumor growth</td>
<td>[29]</td>
</tr>
<tr>
<td>Sodium hyaluronate</td>
<td>Micromoulding 5x5 arrays</td>
<td>5-Aminolevulinic acid</td>
<td>Height: 907.2 ± 20 μm; Base width: 309 ± 16 μm</td>
<td>KB tumor-bearing BALB/c nude mice</td>
<td>In vivo</td>
<td>Reduction of the tumor volume in 56%</td>
<td>[77]</td>
</tr>
<tr>
<td>Polyvinyl alcohol</td>
<td>Micromoulding 10x10 arrays</td>
<td>Doxorubicin</td>
<td>Height: 451.02 ± 8.07 μm; Base width: 161.45 ± 8.49 μm</td>
<td>N.A.</td>
<td>Proof-of-concept</td>
<td>Increased doxorubicin delivery across the skin</td>
<td>[86]</td>
</tr>
<tr>
<td>Polyvinyl pyrrolidone</td>
<td>Micromoulding 10x10 arrays</td>
<td>Doxorubicin and indocyanine green-loaded mesoporous silica nanoparticles</td>
<td>Height: 600 μm; Base width: 200 μm</td>
<td>MG-63 tumor-bearing BALB/c nude mice</td>
<td>In vivo</td>
<td>Reduction of the tumor volume in 50% and 80% of tumor cells necrosis</td>
<td>[80]</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>Micromoulding 20x20 arrays</td>
<td>Gold nanorods and doxorubicin</td>
<td>Height: 480 μm; Base width: 300 μm</td>
<td>A431 tumor-bearing BALB/c nude mice</td>
<td>In vivo</td>
<td>Complete eradication of the tumor</td>
<td>[82]</td>
</tr>
<tr>
<td>Cellulose</td>
<td>Micromoulding 9x9 arrays</td>
<td>Lipid-coated cispatin nanoparticles</td>
<td>Height: 800 μm; Base width: 400 μm;</td>
<td>HNSCC cell lines FaDu, CAL27, SCC 15 and Female BALB/c nude mice</td>
<td>In vivo</td>
<td>Stalled tumor progression and increased skin safety</td>
<td>[27]</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>Micromoulding 10x10 arrays</td>
<td>Gold nanocubes and doxorubicin</td>
<td>Height: 450 μm; Base width: 200 μm;</td>
<td>B16F10 tumor-bearing C57 mice</td>
<td>In vivo</td>
<td>Increased mice survival and impaired tumor growth</td>
<td>[84]</td>
</tr>
<tr>
<td>Zein</td>
<td>Micromoulding 6x6 arrays</td>
<td>Tamoxifen and gemcitabine</td>
<td>Height: 965 ± 23 μm; Base width: 363 ± 15 μm</td>
<td>N.A.</td>
<td>Proof-of-concept</td>
<td>Increased skin permeability to the drugs</td>
<td>[23]</td>
</tr>
<tr>
<td>Stainless steel</td>
<td>Wet etching 57 tips</td>
<td>5-Aminolevulinic acid</td>
<td>Height: 700 μm;</td>
<td>A-20 tumor-bearing BALB/c mice</td>
<td>In vivo</td>
<td>Reduction of the tumor volume in 70%</td>
<td>[87]</td>
</tr>
<tr>
<td>Polyvinyl alcohol, polyvinyl pyrrolidone, and polycaprolactone</td>
<td>Micromoulding 9x9 arrays</td>
<td>Doxorubicin and LaB6 nanomaterials</td>
<td>Height: 600 μm; Base width: 300 μm;</td>
<td>4T1 tumor-bearing SCID mice</td>
<td>In vivo</td>
<td>Complete eradication of the tumor and</td>
<td>[28]</td>
</tr>
</tbody>
</table>
5. Conclusions and future perspectives

Despite all the efforts, the systemic anticancer therapeutic approaches still present several disadvantages such as the excessive complexity to be adequately applied in the clinic and undesired interactions with healthy tissues. Therefore, the development of macroscale delivery systems that can be locally implanted on the tumor tissue and by-pass all the hurdles of the systemic delivery have been showing promising results for cancer therapy. As an emerging delivery device, the microneedles can be used to efficiently deliver both small and macromolecules, such as chemotherapeutics, proteins, and genetic material, or even nanoparticle-based anticancer therapies. Moreover, the microneedles can be fabricated using diverse materials and different methods allowing a great diversity on the device design and therapeutics release. As highlighted in this review, microneedle-based devices have the potential to deliver simultaneously combinations of drugs and photodynamic agents, nanoparticles and drugs, genetic material and antibodies, and others. Nevertheless, despite the great potential of microneedles for improving the efficacy of the commonly applied anticancer therapies, several comprehensive studies are still necessary to fully characterize the behavior of these microscale drug delivery devices on human therapeutic applications. Moreover, the successful application of these therapies is dependent on several
important parameters, such as the timing for combining the action of these different strategies, the therapeutics concentration, the exposure time, and the release sequence. In this field, the combination of microneedles with layer-by-layer production techniques can be a highly promising and straightforward approach to allow the production of complex and hierarchically organized structures. Further, these microneedle devices carry the potential for improving not only the efficacy of anticancer therapies but also improve the transdermal drug delivery in the context of other health disorders.

Acknowledgements

This work was supported by FEDER funds through the POCI – COMPETE 2020 – Operational Programme Competitiveness and Internationalisation in Axis I – Strengthening research, technological development, and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT – Foundation for Science and Technology (Project UID/Multi/00709/2013). The funding from CENTRO-01-0145-FEDER-028989 and POCI-01-0145-FEDER-031462 are also acknowledged. André F. Moreira, Elisabete C. Costa, and Sónia P. Miguel acknowledge for their individual Ph.D. fellowship from FCT (SFRH/BD/109482/2015, SFRH/BD/103507/2014, and SFRH/BD/109563/2015, respectively). The funders had no role in the decision to publish or in the preparation of the manuscript.

Conflict of interest

The authors declare no financial or commercial conflict of interest.

References


