Brief/Technical Note

Characterization of Nanoporous Surfaces as Templates for Drug Delivery Devices

Ashish Rastogi,¹ Tanushree Bose,¹ Marc D. Feldman,^{2,3} Devang Patel,² and Salomon Stavchansky^{1,4}

Received 3 August 2009; accepted 5 October 2009; published online 30 October 2009

KEY WORDS: cyanoacrylate; drug delivery; methyl orange; nanopores; volume calculation.

INTRODUCTION

While the earlier research in drug delivery has been focused on development of drugs, present methodologies target development of the delivery device itself (1–3). Implantable drug delivery devices offer various advantages such as maintenance of therapeutic blood levels, improved patient compliance, and improved safety (4). Nanorobots capable of treatment, prophylaxis, and diagnosis represent the next generation of drug delivery devices (5,6). Carbon nanotubes have been developed to seek and destroy tumor cells (7). Recently, Martin *et al.* tailored the width of microfabricated nanochannels to solute size to control diffusion kinetics of macromolecules (8).

In this study, a nanoporous metal surface has been characterized for purpose of drug delivery. Drug loading into nanopores can be achieved using solutions, colloidal solutions, or polymer–drug systems. Early reports in literature have suggested use of lithography techniques for fabrication of nanostructures (9,10). Silicon wafers are coated with a combination of gold and silver. Gold is selected as a suitable surface material because it has been extensively used in development of novel nanodiagnostic tools due to its mechanical stability and biocompatibility (11–13). In photolithography, a photomask is used to transfer the pattern onto the wafer and a layer of photosensitive polymer (photo-resist) is applied using spin coating technique. The wafers are then exposed to ultraviolet light. The mask protects the portion of the wafer it covers, whereas the uncovered part gets etched by light. Silver, which is used as a sacrificial material, is precipitated out leaving, nanopores behind.

In the present study, standard metric techniques, namely scanning electron microscope (SEM) and atomic force microscope, were used for characterization of the nanopores. The dimensions of the nanopores were estimated, and the measurements were used to determine the total volume of pores available for drug loading. For purpose of estimation, a bare metal stent was used as a reference. Hence, the volume of nanopores if they were built on a stent surface was calculated.

In this study, nanopores were loaded using methyl orange, pH indicator (14). Poly (2-octyl cyanoacrylate) was used as the polymer matrix because of its strong adhesive properties, biocompatibility, and as a drug carrier (15–17). It is also approved by FDA and is being currently used as a tissue adhesive (18). The role of the polymer here is to act as a sealing film formation to limit the rapid release of methyl orange from the nanopores.

MATERIALS AND METHODS

Materials

Bare silicon wafers, gold-coated silicon wafers, and nanoporous gold-coated silicon wafers were obtained from Nanomedsystems, Charlottesville, VA, USA (formerly Setagon, Inc.). A bare metal stent (PalmaZ-Schatz® Balloon-expandable stent, size = 15 × 3.0 mm) was obtained from The University of Texas Health Sciences Center, San Antonio, TX, USA. Methyl orange was obtained from Fisher Scientific. BAND-AID® Brand Liquid Bandage containing 2-octyl cyanoacrylate as the active ingredient was obtained from the local pharmacy store.

Surface and Dimensional Analysis of Nanoporous Wafers

The bare metal stent's length, width, and thickness were estimated using a Hitachi S-4500II SEM. Three wafers from each group of wafers were selected, and their surface morphology was compared using the SEM. Dimensional analysis of the nanoporous wafers was performed using SEM and AFM. The AFM topographic images of the

¹ Division of Pharmaceutics, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712, USA.

² Division of Cardiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78229, USA.

³ South Texas Veterans Affairs Health Care System, San Antonio, Texas 78229, USA.

⁴ To whom correspondence should be addressed. (e-mail: stavchansky@mail.utexas.edu)

Fig. 1. **a** Silicon wafer (magnification, $\times 500,000$). The silicon serves as a start material for the production of nanopores. **b** The silicon wafer is coated with gold by dipping method (magnification, $\times 200,000$). **c** Nanopores are produced on the gold-coated silicon wafer using photolithography process. The pores are non-homogenous but elongated in shape (magnification, $\times 200,000$)

nanoporous wafers were analyzed using the particle analysis and the section analysis commands, yielding length, width, area of the pores, and depth of the pores, respectively. The software used in AFM was Nanoscope 5.12b48 and the Cantilever used was of 300 kHz frequency.

Drug Loading of Nanoporous Wafers

A thin layer of an ethanolic solution of methyl orange was applied onto the surface, and ethanol was evaporated using a heat gun. A drop of 2-octyl cyanoacrylate solution was

then applied onto the wafer followed by the addition of a drop of water for polymerization of the monomer. The weight of methyl orange and cyanoacrylate loaded was estimated gravimetrically by weighing the wafers after each step.

Drug Release Study

The *in vitro* drug release study in 10 ml of distilled water was performed in a non-stirred environment (19). After each 24-h period, aliquots were collected and replenished with fresh distilled water to assure sink conditions. The collected samples were retained for absorbance measurements at 464 nm against a blank.

RESULTS AND DISCUSSION

Dimensional Analysis of Nanopores

The SEM pictures of the three distinct group of wafers, namely silicon wafer, gold-coated silicon wafer, and nanoporous gold coated silicon wafers, are illustrated in Fig. 1a–c, respectively. The length and width of nanopores can be measured manually from SEM pictures. The depth was estimated using AFM as illustrated in the topographic images (Fig. 2a, b). Table I represents the cumulative

Fig. 2. The figure shows the nanopores as analyzed using AFM (**a**). The section analysis of $1\text{-}\mu\text{m}^2$ area of the wafer using AFM shows top view of the nanopores as indicated by the *brown region* and was used to analyze the depth of the pores (**b**). The particle analysis of $1\text{-}\mu\text{m}^2$ area of the wafer using AFM shows horizontal view of the nanopores as indicated by the *red region* and was used to analyze the length, width, and area of the pores in the wafers

Table I. Statistical Analysis of Nanoporous Wafers using Dimensions Obtained from AFM ($N=5$)

	Area (nm ²)	Diameter (nm)	Length (nm)	Width (nm)	Depth (nm)
Mean	1,317	27.3	60.0	14.9	26.9
SD	219	4.9	10.9	2.32	3.8
% CV	16.6	17.9	18.2	15.6	14.1

statistical data of the five nanoporous wafers. The SEM pictures (Fig. 1c) suggest the pores to be non-homogenous in shape. We assumed the majority of the pores to be cylindrical in shape. Hence the volume was estimated using the equation:

$$V = AD \quad (1)$$

Where V , A , and D are the volume, area, and depth of the pores. Here, the pore area determined by the AFM studies is the area of the pore surface and not the area of the interior surface of the pore channel. The volume of nanopores was calculated as $3.55 \times 10^4 \text{ nm}^3/\mu\text{m}^2$ area of wafer surface or $3.55 \times 10^4 \text{ nm}^3/\mu\text{m}$ length of wafers.

Surface Analysis of a Stent and Estimation of Volume of Pores on Its Surface

A commercially available bare metal stent, which is made up of 87 mini cylindrical rods, was used for calculations (Fig. 3a). The length, width, and thickness of the rods were estimated using SEM pictures (Fig. 3b). The stent is cylindrical in shape with open ends. Figure 3b indicates the length of the cylindrical rods as $1.3 \times 10^3 \mu\text{m}$. Hence,

$$\begin{aligned} \text{Total length available on stent for fabrication of nanopores} \\ = 87 \times 1.3 \times 10^3 \mu\text{m} = 0.113 \text{ m} \end{aligned} \quad (3)$$

Hence, total volume of pores available on entire length of the stent can be given by:

$$\begin{aligned} &= (\text{Volume of pores}/\mu\text{m}) \times (\text{Total length of stent}) \\ &= (3.55 \times 10^4 \text{ nm}^3/\mu\text{m}) \times (0.113 \text{ m}) \\ &= 4.01 \times 10^9 \text{ nm}^3, \text{ which is the estimated volume available for drug loading} \end{aligned}$$

Drug Release Study

The aim of this study was to characterize the drug loading capacity of nanopores. The drug loading data, which are summarized in Table II, indicates that a uniform w/w ratio of methyl orange and cyanoacrylate, 0.70 ± 0.04 , was applied to the wafers. The average cumulative percentage release of $88.1 \pm 5.0\%$, equivalent to $220 \pm 97 \mu\text{g/day}$ of methyl orange, was obtained for first 7 days (Fig. 4). Certain patients may be hypersensitive to polymers (20), in such

Fig. 3. **a** A skeleton design of a bare metal stent composed of 87 mini cylindrical rods. **b** SEM pictures of the stent were used to estimate the length of the cylindrical rods. The stent design and the reference stent were used as a reference to calculate the pore volume of the nanopores

cases, biodegradable or bioabsorbable polymers may be used instead of poly-cyanoacrylates.

CONCLUSION

Gold proved to be an effective material for the fabrication of nanopores, which can be fabricated in different patterns and by different techniques. SEM and AFM have proven to be useful tools in analyzing nanofeatures. Poly (2-octyl cyanoacrylate) can be successfully used to prolong methyl orange release from nanoporous surface, metals, and probably other surfaces. The technique can be extended to hydro- and lipophilic drugs. The result of this study suggests the possible use of nanoporous surfaces for extended drug release.

Table II. Drug Loading Data ($N=3$)

	Weight of wafer in mg	Weight of methyl orange loaded (D), in mg	Weight of cyanoacrylate added (C), in mg	Ratio of D/C
Wafer 1	34.0	2.5	3.6	0.7
Wafer 2	30.6	1.5	2.4	0.6
Wafer 3	33.9	1.2	1.9	0.6

Fig. 4. A cumulative percentage release profile of methyl orange from cyanoacrylate polymer matrix. Values are presented as mean with standard deviation ($n=3$)

REFERENCES

- Gao Y, Gu W, Chen L, Xu Z, Li Y. A multifunctional nano device as non-viral vector for gene delivery: *in vitro* characteristics and transfection. *J Control Release*. 2007;118(3):381–8.
- Kogure K. Development of a novel artificial gene delivery system multifunctional envelope-type nano device for gene therapy. *Yakugaku Zasshi*. 2007;127(10):1685–91.
- Kogure K, Akita H, Yamada Y, Harashima H. Multifunctional envelope-type nano device (MEND) as a non-viral gene delivery system. *Adv Drug Deliv Rev*. 2008;60(4–5):559–71.
- Langer R. Implantable controlled release systems. *Pharmacol Ther*. 1983;21(1):35–51.
- Lou M, Jonckheere E. Magnetically levitated nano-robots: an application to visualization of nerve cells injuries. *Stud Health Technol Inform*. 2007;125:310–2.
- NanotechWeb. <http://nanotechweb.org/cws/article/lab/32077>. Accessed January 15 2009.
- Kam NW, O'Connell M, Wisdom JA, Dai H. Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction. *Proc Natl Acad Sci USA*. 2005;102(33):11600–5.
- Martin F, Walczak R, Boiarski A, Cohen M, West T, Cosentino C, *et al*. Tailoring width of microfabricated nanochannels to solute size can be used to control diffusion kinetics. *J Control Release*. 2005;102(1):123–33.
- Park M, Harrison C, *et al*. Block copolymer lithography: periodic arrays of $\sim 10^{11}$ Holes in 1 Square Centimete. *Science*. 1997;276(5317):1401–4.
- Quake SR, Scherer A. From micro- to nanofabrication with soft materials. *Science*. 2000;290(5496):1536–40.
- Jain KK. Nanodiagnostics: application of nanotechnology in molecular diagnostics. *Expert Rev Mol Diagn*. 2003;3(2):153–61.
- Liang KZ, Qi JS, Mu WJ, Chen ZG. Biomolecules/gold nano-wires-doped sol-gel film for label-free electrochemical immuno-assay of testosterone. *J Biochem Biophys Methods*. 2008;70(6):1156–62.
- Sun J, Graeter SV, Yu L, Duan S, Spatz JP, Ding J. Technique of surface modification of a cell-adhesion-resistant hydrogel by a cell-adhesion-available inorganic microarray. *Biomacromolecules*. 2008;9(10):2569–72.
- Henzla J, Bredowb T, *et al*. Irreversible isomerization of the azobenzene derivate methyl orange on Au(111). *Chemical Physics Letters*. 2007;435(4–6):278–82.
- Eskandari MM, Ozturk OG, Eskandari HG, Balli E, Yilmaz C. Cyanoacrylate adhesive provides efficient local drug delivery. *Clin Orthop Relat Res*. 2006;451:242–50.
- Huang CY, Lee YD. Core-shell type of nanoparticles composed of poly[(n-butyl cyanoacrylate)-co-(2-octyl cyanoacrylate)] copolymers for drug delivery application: synthesis, characterization and *in vitro* degradation. *Int J Pharm*. 2006;325(1–2):132–9.
- Vauthier C, Dubernet C, Chauvierre C, Brigger I, Couvreur P. Drug delivery to resistant tumors: the potential of poly(alkyl cyanoacrylate) nanoparticles. *J Control Release*. 2003;93(2):151–60.
- Eaglstain WH, Sullivan TP, Giordano PA, Miskin BM. A liquid adhesive bandage for the treatment of minor cuts and abrasions. *Dermatol Surg*. 2002;28(3):263–7.
- Price RR, Patchan M. Entrapment and release characteristics of 2-methoxynaphthalene from cylindrical microstructures formed from phospholipids. *J Microencapsul*. 1993;10(2):215–22.
- Nebeker JR, Virmani R, Bennett CL, Hoffman JM, Samore MH, Alvarez J, *et al*. Hypersensitivity cases associated with drug-eluting coronary stents: a review of available cases from the Research on Adverse Drug Events and Reports (RADAR) project. *J Am Coll Cardiol*. 2006;47(1):175–81.