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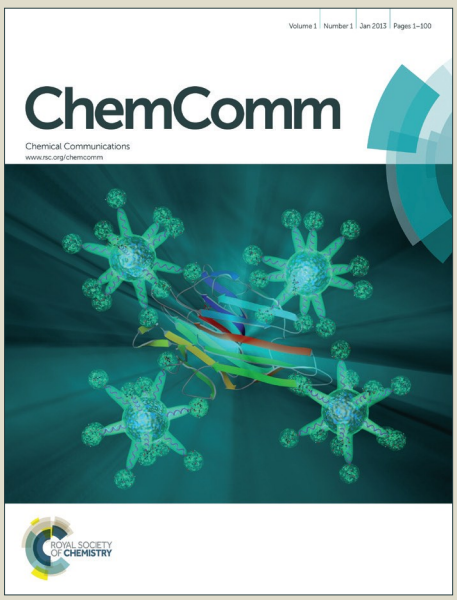

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Journal Name

ARTICLE

Reactions in ultra-small droplets by tip-assisted chemistry

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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The confinement of chemical reactions within small droplets has received much attention in the last few years. This approach has been proved successful for the in-depth study of naturally occurring chemical processes as well as for the synthesis of different sets of nanomaterials with control over their size, shape and properties. Different approaches such as the use of self-contained structures or microfluidic generated droplets have been followed over the years with success. However novel approaches have emerged during the last years based on the deposition of femtolitre-sized droplets on surfaces using tip-assisted lithographic methods. In this feature article we review the advances made towards the use these ultra-small droplets patterned on surfaces as confined nano-reactors.

Introduction

Femtolitre chemistry has arisen in the last few years as an exciting approach to synthesize nanoscale materials in a highly controlled manner. In combination with lithographic and micro/nano-fabrication techniques, it has opened the door to the creation of large and dense arrays of nano-reaction vessels for high-throughput screening, combinatorial chemistry/biology or chemical synthesis.[1] Beyond the need for nanostructured materials, there are several other scientific motivations to conduct chemistry at this scale. A femtolitre (fL= 10^{-15} L, 1 μm^3) is approximately the volume of a bacterial cell, and the ultimate chemistry of life takes place at this ultra-small scale that ranges from picolitres (pL=10^{-12} L, 10 μm^3) to attolitres (aL=10^{-18} L, 100 nm^3).[2] Reproducing these highly crowded and confined conditions is therefore essential to understand their effect on the thermodynamics and kinetics of confined biological and chemical reactions. This need has fuelled the development of a wide range of synthetic nanostructured (bio)environments, including cell-like compartments for encapsulating biochemical reactions, nanostructured containers for fundamental studies of diffusion, or nanofabricated topological features that regulate biomolecular interactions.[3] In addition, the study of naturally occurring chemical processes in confined conditions may shed light onto relevant fields such as the origin of life or atmospheric aerosols that are still poorly understood.[4]

Apart from the fundamental studies of (bio)chemical processes at the nanoscale, applied chemistry has also benefited from the advances made in femtolitre chemistry. In the synthesis of nanomaterials, droplets can act as templates to control parameters such as particle size and shape or surface texture[5] and thus, to tune morphology-size-property relationships. So far, different approaches have been followed to generate miniaturized droplet-based reactors. The most extended procedures make use of self-contained structures (like droplet emulsions, liposomes, micelles and protein cages) or microfluidic-generated droplets. An alternative methodology consisting in depositing small droplets on surface using tip-assisted lithographic methods has emerged in the last few years. With this approach the droplets can be used as confined reactors to precisely control the position of the resulting materials on the substrate. The interest of this methodology relies in the reduction of the number of steps needed to pattern functional materials on a surface, as the synthesis and patterning processes are performed simultaneously.

In this feature article we review recent research involving femtolitre-sized reactions. The methodologies based on self-contained structures and microfluidic-generated droplets have already been extensively reviewed[2,6,7] and are only briefly addressed here. Instead, we mainly concentrate on the emerging tip-assisted methodologies and the materials obtained directly in femtolitre-sized droplets deposited on surfaces.

Confined reactions in self-contained structures and microfluidic channels

Reactions confined in self-assembled containers

Water-in-oil emulsions are metastable colloids that represent the simplest example of nanocontainers. They are composed of two immiscible fluids, one being dispersed in the other in the shape of femtolitre-sized dropets.[8] These structures have been extensively used to confine biochemical reactions such as the polymerase chain reaction (PCR)[9] and other processes like cell-free protein expression.[10] The resemblance of the lipid bi-

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layer wall of liposomes to cell membranes has favoured their consideration as "artificial cells"[11] and arrays of lipid vesicles were suggested as libraries for the simultaneous screening of multiple analytes.[12] Moreover, the permeability and stability of the lipid bilayer can be tuned through external stimuli such as electric pulses or temperature changes to trigger the reactions occurring inside the liposomes.[13,14] The use of capsosomes, liposomes embedded within polymeric capsules, allowed to perform coupled and parallel enzymatic reactions in confined conditions by loading the liposomes with different enzymes.[15]

These supramolecular organic templates have also been extensively used for the synthesis of multiple nanoscale solids, from metallic and ceramic nanoparticles[5,16–20] to hybrid composites[21] or metal-organic particles.[22] The strategy consists in mixing two microemulsions (direct or reverse), one containing the metallic precursor and the other one the so-called precipitating agent (Figure 1). Among all the different nanomaterials synthesized in this way, nanoscale metal-organic materials and coordination polymers have especially benefitted from this methodology, as it allowed to radically improve the control over the size, shape and crystallinity of the resulting particles.[23] Indeed, since the pioneering work by Mann and co-workers on the synthesis of Prussian blue nanoparticles in reverse microemulsions,[24] an increasing amount of reports have appeared that employ this method to obtain and control the shape and size of Prussian blue analogs,[25–28] metal-organic frameworks (MOFs),[29–32] or spin-crossover polymers[33–36] among others. Microemulsions have also been used as nanoreactors to produce polymeric nanoparticles[37–40] and protein nanoparticles.[41,42]

Beyond synthetic assemblies, natural nanoarchitectures such as viral capsids and other protein cages have also been used as nanoreactors.[7,43,44] The main advantage of the use of natural nano-containers in comparison to synthetic supramolecular assemblies relies on their improved monodispersity and robustness as well as the broad range of sizes available and the possibility to easily functionalize the protein shell.[45,46] For these reasons, protein-based nanocontainers have been used as templates for the synthesis of nanoscale inorganic materials

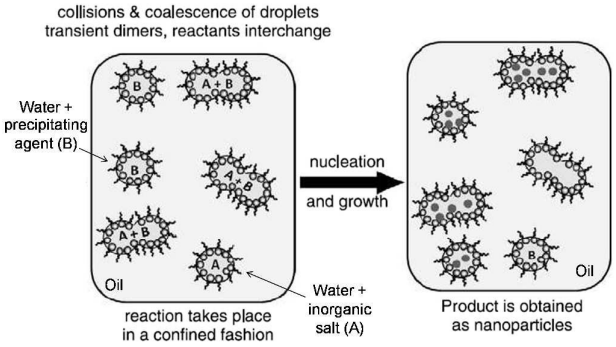


Figure 1. Schematic representation of the synthesis of nanoparticles using water-in-oil emulsions. For purely inorganic materials, the precipitating agent (B) is usually a reducing agent such as $NaBH_4$; in the case of metal-organic particles, (B) corresponds to the organic ligand(s). Reproduced with permission from ref. 23.

such as metal and metal-oxide nanoclusters,[47,48] as well as to perform and study confined enzymatic reactions.[49–51] Self-assembling peptide polynanoreactors have also been described and applied to the synthesis of silver nanoparticles.[52]

Reactions confined in droplets generated in microfluidic channels and micro/nano-wells

Microfluidic devices are commonly used to generate and mix droplets under highly controlled environments. This high level of control is achieved thanks to the generation of microfluidic droplets with perfectly controlled and uniform size at the cross-stream flow of two immiscible liquids.[6,53] Due to that, microfluidic generated droplets are highly reproducible synthetic environments that, in turn, provide highly reproducible conditions and materials. An important added value of performing femtolitre chemistry in microfluidic devices is the reduced amount of reagents and solvents that are consumed. This is highly important when performing screening studies using precious materials. For this reason, crystallization of proteins and pharmaceuticals[54–56] as well as screening of organic synthetic reactions have been performed using microfluidic tools.[57–59]

Plenty of examples on the use of microfluidics for confined biochemical reactions can be found in the literature, going from enzyme kinetics[60] to protein expression[61,62] and single-cell studies.[63,64] However, probably the area in which this approach has offered more innovative advances is the synthesis of micro-/nanoparticles,[65] as it allows for a precise control of the size distribution.[66,67] As an example, gold nanoparticles[68] or nanorods with tunable aspect ratio were obtained in microfluidics-generated droplets.[69] Other technologically relevant inorganic nanocrystals such as CdSe quantum dots,[70] superparamagnetic iron oxide nanoparticles (SPIONs),[71] silver,[72] zeolites[73] or core-shell nanostructures[74] as well as polymeric microcapsules[75] and solid particles[76] have also been obtained in this way. Additionally, the confined synthesis of a series of MOFs and core-shell MOF composites using microfluidic tools has been recently described in two almost simultaneously released papers (Figure 2).[77,78] Both research groups reported that the confinement of the reaction afforded highly homogeneous crystals whilst significantly reducing the reaction time.[79]

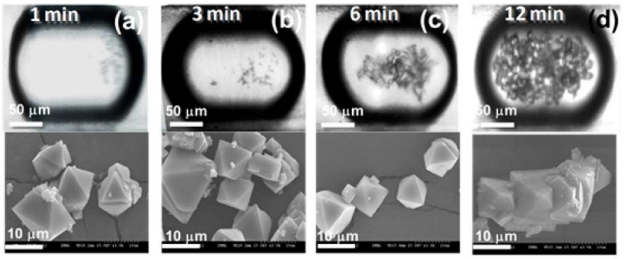


Figure 2. Optical and SEM micrographs of HKUST-1 crystals obtained in microfluidic droplets with increasing reaction times. Reproduced from with permissopn from ref. 77.

Finally, in parallel to microfluidics, homogeneous arrays of nanocontainers fabricated on surfaces using lithographic techniques or optical fiber bundles have been used to obtain large arrays of ultra-small reaction vessels.[80–82] One of the most important achievements in this field was the fabrication of the so-called zero-mode waveguides,[83] which have allowed the observation of single-molecule dynamics of increasingly complex biological systems at high concentrations.[3]

Tip-assisted lithography: an introduction

Direct write AFM-assisted lithography (also referred to as Scanning Probe Lithography (SPL), AFM lithography or tip-assisted lithography) is a high-resolution lithographic technique that uses a sharp tip to pattern nano-to-microscale features on a surface. It resembles a normal writing process where the AFM tip is used as "pen", a solid state substrate acts as "paper" and a solution containing the material(s) as "ink".[84] The molecules or nanostructures acting as inks are first coated on the tip and then transported to the surface by engaging and traversing the tip over the substrate in the form of the desired pattern. Although any AFM probe can be theoretically employed, typically specially designed probes (commonly referred to as "pens") are used, which show pyramidal shapes and tip radii ~15 nm.

The process of transferring molecules from an AFM tip to a substrate was first described in 1995 by Jaschke and Butt,[85] who deposited aggregates of octadecanethiol (ODT) in irregular-shaped structures with homogeneous height of 1.2 nm onto freshly cleaved mica. A few years later, in 1999, Mirkin and co-workers organized alkanethiol molecules on Au surfaces forming well-defined SAMs with excellent resolution (down to 12 nm)[86,87] and obtained multi-component patterns composed of different alkanethiols, reducing the separation to only 5 nm.[88,89] These results led to the invention of a commercialized process called Dip-Pen Nanolithography® (abbreviated as DPN®) that became a registered trademark of NanoInk, Inc. (Chicago, IL). In the following years the technique became increasingly popular[90,91] and, since then, a myriad of materials have been successfully structured in a wide variety of substrates using DPN.[92–104]

The scalability of AFM-assisted lithography has always been questioned due to the low throughput of the technique, motivated by its inherent serial writing nature. Because of that, it has been considered a technique restricted to proof-of-concept studies and basic science. In order to expand the limits of the technique, other derived tip-assisted lithographies such as Polymer Pen Lithography (PPL) have appeared.[105] This young technique combines the feature size control of direct write AFM lithography with the large-area printing capability of micro-contact printing. The writing tool consists of an array of up to 11 million elastomeric pyramids (typically polydimethylsiloxane, PDMS) that are coated with the inks and brought into contact with the surface to create patterns over large areas.[106–109] The appearance of this and other related massively parallel cantilever-free printing tools[110–112] has solved the main inconvenient of direct-write AFM assisted lithography by turning it into a parallelized process.

On the other hand, the main advantage of direct write AFM-assited lithography in comparison with other structuration techniques is that it allows for the precise positioning of materials under environmental conditions onto virtually any substrate without the need of previous surface or material modification.[101] Due to that, this technique has been highly valued for patterning biological entities such as proteins,[113–117] oligonucleotides[118] or living cells.[119] Moreover, it is a non-destructive technique that can be used on fragile and soft surfaces like polymers,[120] graphene[121–123] or living tissues.[99,124,125] Also, direct write AFM-assisted lithography is ideal to be used in fabrication processes of small devices where the last step consists in positioning valuable functional materials on specific areas of a solid support. For instance, our group deposited a diversity of magnetic materials (ferritin-based CoO nanoparticles, Mn_{12} single-molecule magnets and Co nanoparticles) on the most sensitive areas (as small as 1 μm^2) of superconductive sensors to enhance their sensitivity without damaging any of the components of the device.[123,126–129]

According to the ink's nature, direct write AFM lithography experiments can be categorized into two main types: dry and liquid.[102] In the classic (dry) methodology (Figure 3a), the AFM tip is functionalized by small molecules that are transported to the substrate by diffusion through the water meniscus that is formed due to capillary condensation under ambient conditions.[130,131] This procedure was originally developed for the deposition of alkanethiols,[103] but it has extended to more complex inks such as nanoparticles,[132] biomolecules[117,133] and materials supported in matrix carriers.[111]

A completely different methodology consists in dipping the tip in an ink solution for a given time and immediately using it before the solvent evaporates.[134] In this case, the ink is patterned on the substrate by delivering less than femtoliter

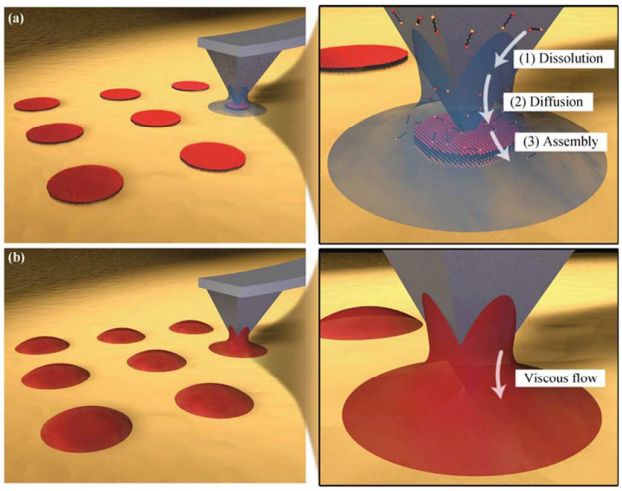


Figure 3. Schematic representation of the writing process in AFM-assisted lithography. (a) Schematics of the classic (dry) procedure in which a soluble small molecule ink diffuses through the water meniscus that forms at the point of contact. (b) Representation of the deposition of femtolitre-sized droplets of a solution directly on the surface. Reproduced with permission from ref. 135.

droplets of the solution (Figure 3b). After the patterning, the solvent evaporates and motifs of the materials in the solid state are obtained. The origin of this methodology lies at the need to pattern materials and nano-objects that do not diffuse easily (or do no diffuse at all) through the water meniscus. However, in the recent years some researchers have envisaged the possibility of using the femtolitre-sized droplets deposited on surfaces as miniaturized vessels where reactions can be performed in an extremely small scale. Although this methodology is only at its infancy stages, several examples can be already found in the literature, as summarized next.

Chemistry within femtolitre droplets deposited by tip-assisted lithography

In the following section we thoroughly review the existing publications on the use of tip-assisted lithographic methods to perform confined reactions on surfaces. For the sake of simplicity we have classified chemistry within femtolitre droplets deposited on surfaces into three different categories, as represented in Figure 4): I) reaction between the ink components and the substrate (Figure 4a), II) reaction of the components already contained in the droplets and delivered in a single step (Figure 4b) and III) mixture of reagents on surface by sequential delivery of solutions containing the different reagents (Figure 4c). The approaches and examples of each one of them are described in detail next.

Approach I: reaction between materials contained in delivered droplets and the target surface.

Out of the three, this is the approach for which a larger amount of examples have been described. It consists in the transformation of the delivered materials upon reaction with some specific groups on the surface. One strategy uses the redox properties of the substrate to *in situ* transform the patterned materials and obtain metallic nanostructures.

For example, in 2001[136] the reducing capability of an activated Si substrate was used to reduce Au(III) precursors deposited through an AFM tip and form metallic gold patterns. Later on, it was demonstrated that Au and Pt motifs could be obtained by the same process on untreated Ge(100) substrates.[137] More recently, the reducing capability of single-walled carbon nanotubes (SWCNTs) was used to deposit gold seeds on their surface by drawing lines over them with an AFM tip coated with $HAuCl_4$.[138] Although very useful, the applicability of this method is restricted to noble metal precursors that are easily reduced and the use of compatible substrates.

Oppositely, a fairly common example of tip-mediated localized reaction consists in the reaction of at least one of the ink components with appropriate functionalities present on the substrate to form a covalent bond. This methodology is mainly employed to anchor functional compounds to the surface, so the patterns can resist subsequent washing steps and wet treatments. The first example of this methodology was reported in 2007 and described the anchoring of an azide-functionalized dendron on an alkyne-modified surface[139] through copper-catalyzed azide-alkyne cycloaddition (CuAAC, one of the most common examples of click-chemistry). Following this pioneering work, other reports have appeared employing the same methodology to pattern multiple substrates[140] and a variety of materials, including fluorescent probes or biologically active species.[141] For instance, our group used an amino-terminated surface to covalently anchor and pattern three fluorescent pH-responsive compounds (Oregon Green®, fluorescein and 5-carboxynaphthofluorescein) bearing amino-reactive groups. By using multiple cantilever arrays, large ordered and combinatorial arrays of those optically active molecules were fabricated.[142] Exposure of the patterned surfaces to solutions or gas flows of different pH resulted in reversible changes in the fluorescence signal of the patterned chemosensors (Figure 5).

Of course, massively parallel techniques derived from AFM-assisted lithography have also been used to fabricate femtolitre-sized reactors on surface. For instance, Braunschweig et al. reported the use of Polymer Pen lithography (PPL) for copper-catalyzed click chemistry[143] as

Figure 5. Acid-base reactions of optically active compounds structured on surfaces. (a)

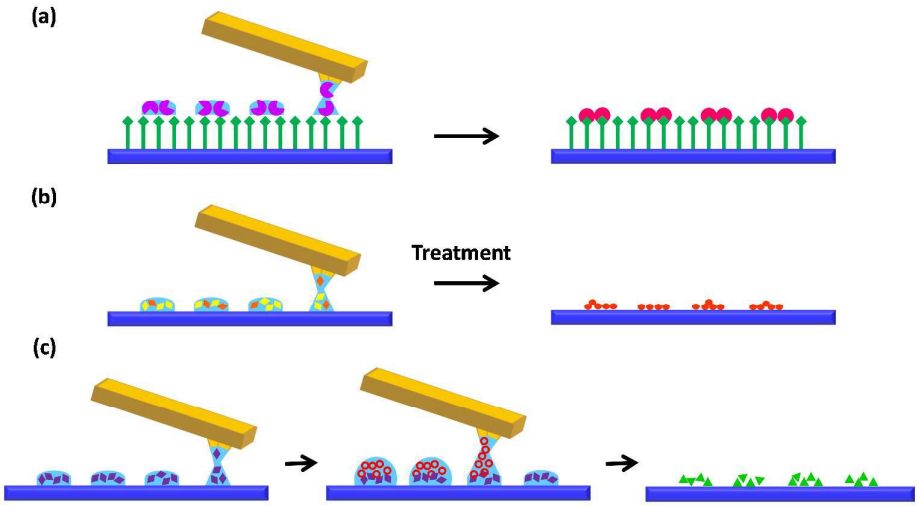
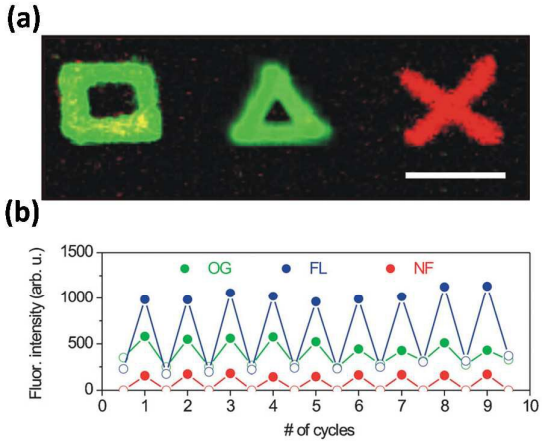


Figure 4. Schematic representation of the different methodologies employed to carry out reactions confined into femtolitre droplets using tip-assisted lithographies. (a) Reaction with the substrate. (b) Transformation of deposited precursors. (c) Mixture of reagents by additive deposition.



carboxynaphthofluorescein (cross) structures; scale bar 5 µm. (b) Average fluorescence intensity of oregon green, fluorescein and 5-carboxynaphthofluorescein dots upon consecutive cycles of exposure to buffer solutions of pH 3.5 (empty circles) and 9.5 (full circles). Reproduced from ref. 142.

well as for the Staudiger ligation[144] to obtain fluorescent (Figure 6) and redox-active patterns and protein recognition platforms anchored to different substrates. More recently, the same researchers used PPL to carry out force-accelerated Diels-Alder reactions on graphene sheets[145]and studied the effect of the applied force on the velocity of a 1,3-dipolar cycloaddition in the absence of copper.[146] Another massively parallel tip-based technique derived from PPL and named Beam Pen Lithography (BPL)[147] was used to produce 3D patterns of polymer brushes by photoinduced radical polymerization.[148] In this occasion the reaction performed inside the fabricated features not only involved anchoring of the material to the surface but also polymerizing the brushes.

Approach II: Reaction between the materials contained in femtolitre droplets.

In this section we address the cases in which femtolitre droplets containing the reaction precursors are patterned on surfaces in order to force the reaction to proceed inside the deposited nanoreactors. In some cases, the reaction is triggered after the patterning by an external stimulus whilst in others it occurs spontaneously.

The precedents to this approach were already settled a few years ago, when our group carried out the assembly and crystallization process of various metal-organic nanostructures confined into femtolitre droplets.[149] In that occasion, crystals of the well-known HKUST-1 ($[Cu_3(BTC)_2]$, BTC= benzenedicarboxylate) and hollow structures of polyoxometallates (POMs) were grown directly on gold surfaces after delivering ultra-small droplets of the soluble precursors on the surface through an AFM tip (Figure 7). A reduction in the volume of the deposited droplets, together with the precise control of solvent evaporation afforded the formation of a single nanostructure per deposited droplet.

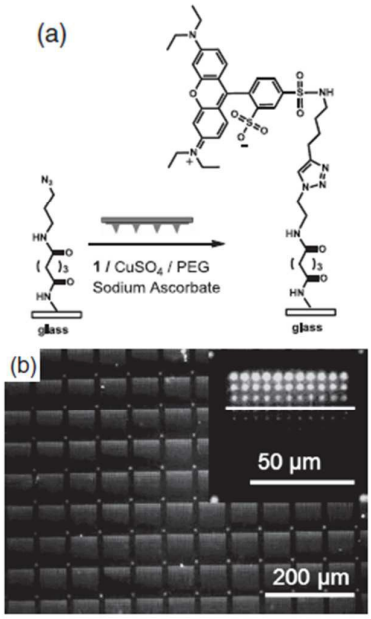


Figure 6. Fluorescent patterns produced by the site-specific copper-catalized azide-alkyne cycloaddition. a) An ink mixture consisting of a fluorescent reagent, PEG, $CuSO_4$, and sodium ascorbate printed onto an azido-terminated glass slide resulted in covalent immobilization of rhodamine. b) Fluorescent microscope image of 11 × 11 dot arrays obtained after patterning the fluorescent reagent with varying dwell times. Inset is a magnified image of one array. Reproduced with permission from ref. 143.

Almost simultaneously, Carbonell and co-workers also described the formation of single HKUST-1 crystals inside femtolitre-sized droplets that were fabricated using Microfluidic Pen Lithography (MPL). This technique uses a microfluidic pen instead of an AFM tip to deliver the solution from a reservoir onto the surface.[150,151] This overcomes one of the main disadvantages of direct write AFM-assisted lithography, which is the depletion of the ink loaded on the cantilever, but at the same time larger droplets are deposited on the surface.

Beyond crystallization and self-assembly processes, the literature provides a fairly wide range of publications that report the performance of chemical reactions on surface after the delivery of a mixture of reagents through an AFM tip. A diversity of metal oxides and sulfides has been obtained using this methodology. For example nanostructures of Al_2O_3, SiO_2 and SnO_2 were fabricated on Si and SiO_2 surfaces using sol-based inks.[152] Briefly, chloride precursors of the metal oxides where brought onto the surface and spontaneously hydrolyzed after getting in contact with the water condensed at the meniscus. In another example a mixture of $Cd(CH_3COO)_2$ and thioacetamide was delivered onto Si surfaces. The thioacetamide gradually releases H_2S upon contact with water and thus CdS spontaneously forms after the ink diffuses to the substrate through the water meniscus.[153] An analogous methodology was employed later on to grow CdS nanoplates on mica.[154] In another example, a heat treatment was used to fabricate barium hexaferrite ($BaFe_{12}O_{19}$) magnetic nanostructures after the delivery of a mixture of $Fe(NO_3)_3$ and $BaCO_3$ in ethyleneglycol on a silicon oxide surface.[155]

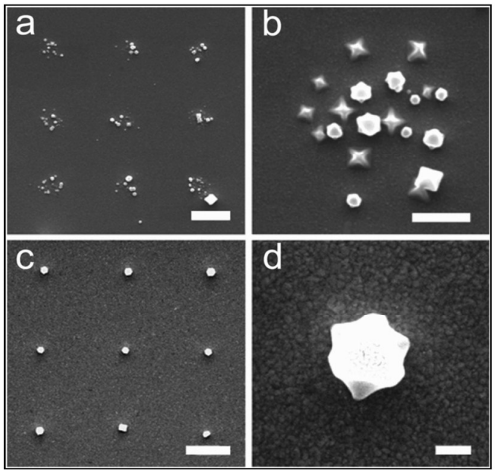


Figure 7. FE-SEM images of HKUST-1 nanocrystals grown inside confined solution droplets deposited by direct write AFM-assisted lithography. (a) Nanoarray; scale bar 2 µm. (b) Nanocrystals grown inside each dot-like feature; scale bar 1 µm. Growth of a single crystal per dot nanoarray viewed from above (c) and at a 45º tilt angle (d); scale bars 2 µm and 200 nm respectively. Reproduced from ref. 149.

The controlled growth of metallic and semiconductor nanoparticles directly on a surface has also received much attention. This is really not surprising given the versatility and interesting properties of these nanoparticles and the vast amount of applications that derive from their assembly and patterning on surfaces.[156–160] Since 2010, the Mirkin group has released several papers using Scanning Probe Block Copolymer Lithography (SPBCL) to obtain a diversity of nanoparticles. In SPBCL, a block copolymer is delivered onto the substrate together with the nanoparticle precursors. The block copolymer acts both as a delivery matrix to facilitate ink transport and as a confined nanoreactor that templates the growth of the nanoparticles induced by plasma reduction.[161] This method was successfully employed to obtain metallic nanoparticles such as Au, Ag or Pd; metal oxide particles like Fe_2O_3 or Co_2O_3, and metal alloys of Au and Ag (Figure 8);[162] in all cases a precise control over the size and position of the particles was achieved. The same technique was used as an additional methodology to obtain CdS quantum dots by exposing the patterned Cd^{2+} precursor to H_2S vapours.[163] Using this methodology, the authors were even able to monitor the growth of the nanoparticles and study the influence of temperature and concentration of the gold precursor on the coarsening process of the particles using in situ Transmission electron microscopy (TEM) experiments.[164] Recently, this technique has been extended to the synthesis of multimetallic nanoparticles made from different combinations of metals (Au, Ag, Pd, Ni, Co and Pt) with precise control over their composition and shape, including the formation of Janus nanoparticles composed of immiscible metals.[165] Additionally, this approach was used to obtain Co_3O_4 nanocluster arrays that further catalyzed the localized growth of carbon nanotubes.[166] PPL has also been used to pattern nanoparticle precursors using ethylene glycol as matrix carrier in order to obtain arrays of single metallic and metal oxide nanoparticle features over extended areas.[167]

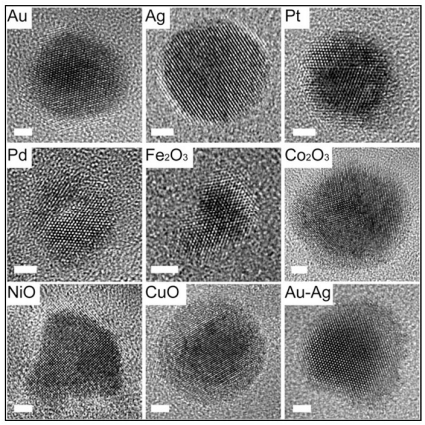


Figure 8. HR-TEM images of different inorganic nanoparticles obtained in confined environments fabricated by SPBCL (Scale bars are 2 nm). Reproduced with permission from ref. 162.

On the other hand, less attention has been payed to the synthesis of purely organic nanomaterials. Our group recently reported the fabrication of the bioinspired polymer polydopamine (PDA) inside femtolitre droplets. For that, a basic solution of dopamine was coated on an AFM tip and immediately delivered on a Si/SiO_2 surface, fabricating femtolitre-sized droplets that acted as confined nanoreactors where the polymerization took place. Rounded PDA features as small as 500 nm in diameter were obtained using this method (Figure 9a). Also, local adhesion measurements and the formation of Ag nanoparticles on the in situ synthesized PDA proved that the structured material retained the adhesive and chemical properties of continuous PDA coatings, confirming the viability of our approach.[168]

More recently, we have reported the synthesis of coordination polymer particles (CPPs) inside droplets deposited on surface.[169] For this we forced the reagents (an aqueous metal salt solution and an organic ligand solution) to mix on the cantilevers during the functionalization of the tips, in order to deliver a just-mixed reacting solution on the target surface. After fabricating dot-like feature arrays of the mixture, the patterned substrates were carefully stored under a highly DMSO saturated atmosphere or high temperature conditions, to achieve the growth of a single particle per deposited droplet (Figure 9b). A similar procedure was used to carry out the miniaturized synthesis of the well-known coordination polymer $[Co(COOCH_3)_2(\mu\text{-}4,4'\text{-bipy})]$ (Co-bipy). Crystalline structures of Co-bipy were obtained in bulk, inside microliter-sized droplets obtained by drop-casting of their soluble precursors and confined into femtolitre droplets delivered onto a Au surface using an AFM tip (Figure 9c). The obtained structures showed different morphologies, corresponding to the crystal growth stage reached in each case (unpublished results).

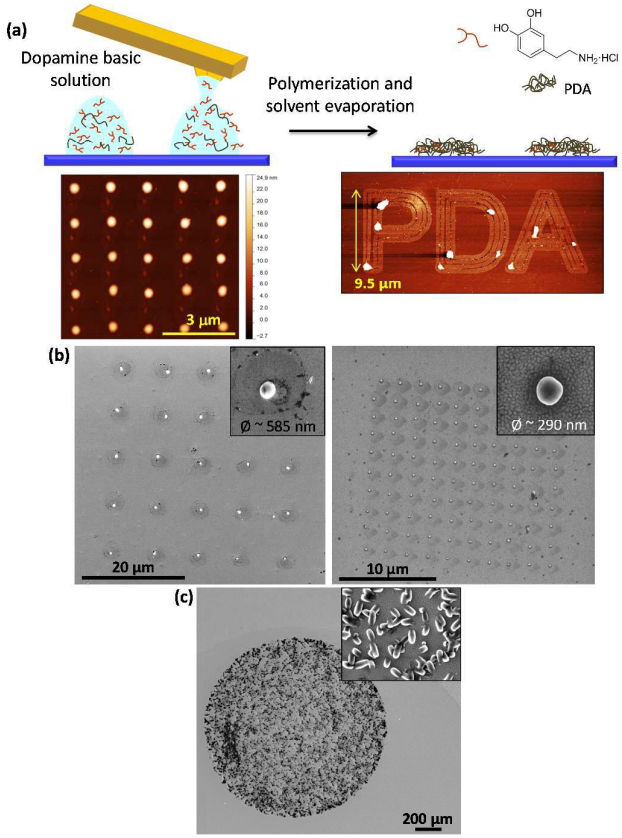


Figure 9. (a) Schematic representation of the experimental procedure followed to structure PDA on surfaces. A freshly prepared basic solution of dopamine was delivered onto the surface using AFM-assisted lithography in the shape of femtolitre-sized droplets where the polymerization took place. Two examples of lithographic patterns obtained with PDA are also shown, a dot-like feature array and microscale letters forming the word PDA. Adapted from ref. 168. (b) Single-particle CPP arrays obtained by delivering a mixture of reagents onto the surface and placing the substrate in a DMSO-saturated atmosphere (left) or keeping it in an oven at 50ºC (right). Adapted from ref. 169. (c) Co-bipy crystalline structures grown after deposition of droplets containing a mixture of the metal ion and the di-topic ligand. Inset: detail of the structures grown inside the droplets (unpublished results).

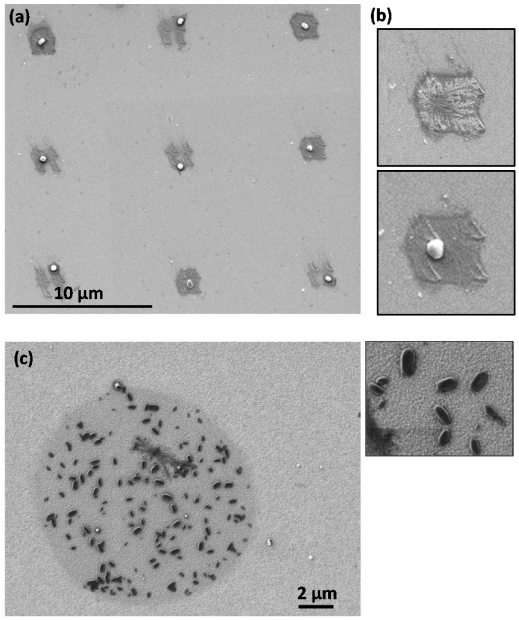


Figure 10. (a) Single CPPs grown inside mixed droplets obtained by depositing each of the inks on the same position of the surface. After the patterning, the substrate was kept in a DMSO-saturated atmosphere for 48 hours and under atmospheric conditions for 48 more hours. (b) Detail of one of the structures in the array. Top: after the first 48 hours a dendritic structure was observed. Bottom: two days later the dendritic structure had evolved to form a single rounded particle. Adapted with permission from ref. 169. (c) Co-bipy crystals obtained after sequential delivery of the reagents and confined reaction and crystallization. Inset: detail of the structures grown inside the droplets (unpublished results).

Approach III: Reactions in femtolitre droplets by sequential addition of reagents.

The last approach reviewed here consists on mixing solutions containing separate reagents by successively placing femtoliter sized droplets of each solution on the same location of the surface. Due to the high complexity and level of precision required, this is by far the less extended methodology of those exposed here. In fact, at the moment of elaboration of this manuscript only two articles reported this procedure in the literature.

The first example was released in 2013 by Maspoch et al. and it describes the use of MPL to mix femtolitre droplets of reagents. The authors reported an extensive and very complete work that included *in situ* acid-base reactions detected by fluorescence microscopy and MOF synthesis and crystallization, including multiplexed arrays of Prussian Blue analogs synthesized by mixing their precursors in situ.[170]

More recently, our group described the use of AFM-assisted lithography to synthesize CPP1 particles on mixed droplets fabricated on surfaces[169] (the synthesis of the same particles by patterning a mixture of the reagents is described in the previous section). Mixed droplets were obtained by delivering each one of the reagent solutions separately on the same position of the surface using a multiple cantilever array. After exposing the substrate patterned with the mixed droplets to a DMSO atmosphere, the growth of a single particle inside each droplet was achieved (Figure 10a). This procedure also allowed to observe the formation of the primitive dendritic structures and their evolution to the final particles (Figure 10b).[169]

Similarly, the sequential delivery of reagents onto a surface in the shape of ultra-small droplets was used to synthesize Co-bipy nanocrystals directly on surface. The coordination reaction between the metal centres and the ditopic ligands was also carried out in femtolitre-sized droplets fabricated on a gold surface and crystalline structures were obtained (Figure 10c). MicroRaman was used to characterize the materials and confirmed the formation of the Co-bipy coordination polymer.

Conclusions

As summarized here, the use of tip-assisted methodologies to perform confined chemical reactions in femtolitre droplets has gained relevance over the last years. To summarize the different examples so far reported, we have grouped them into three main categories. The first and more widely used category groups the different reactions induced upon contact of the delivered droplets with the substrate. Experimentally,

this is the simplest approach out of the three and has been used to perform a variety of chemical reactions including metal ion reduction and click chemistry.

In the second approach, the different reactants are already contained in the solution and react (either spontaneously or induced by an external stimulus) upon deposition of the femtolitre droplets on the surface. The examples found in the literature concerning this methodology are mostly focused on the on-surface synthesis of inorganic nanomaterials, with only a few examples reporting the synthesis of metal-organic systems and purely organic materials.

The third and final approach is definitely the most unusual out of the three, mainly because of the difficulty that implies placing two ultra-small droplets on the same position of a surface with nanometric X-Y resolution while ensuring their effective mixing (Z). However, as the reagents are mixed directly on the surface, it is the only methodology that allows to pattern nanomaterials with fast growth reaction kinetics, opening new application venues.

Overall, several successful examples of tip-assisted chemistry within femtolitre droplets have already been described. Nevertheless, this technology can still be considered at early stages of development. For this reason, and to extend the applicability of this approach, several challenges have still to be faced in the near future. First of all reproducibility of the experiments (especially in the third approach) should increase. Also, a wider range of reactions should be performed in this way; whilst many biochemical processes have been studied in microemulsions and microfluidic-generated droplets, these are not represented still in femtolitre tip-assisted chemistry. Similar considerations are valid to purely organic chemical reactions. However, for that to happen, as important as the synthesis itself, is the development of proper experimental techniques to characterize the materials beyond the imaging-based techniques that have been mostly used until now. This is probably the most important and difficult challenge that we are facing at the moment, and overcoming it will only be possible through the collaboration between scientists of multiple disciplines.

Acknowledgements

M. G. thanks the CSIC for a predoctoral grant (JAEpre). This work was supported by projects MAT2015-70615-R and CTQ2013-41161-R from the Spanish Government and by FEDER funds. ICN2 acknowledges support from the Severo Ochoa Program (MINECO, Grant SEV-2013-0295).

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