Cite this: DOI: 10.1039/xxxxxxxxxx

Developing a tissue glue by engineering the adhesive and hemostatic properties of metal oxide nanoparticles

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Received Date

Accepted Date

DOI: 10.1039/xxxxxxxxxx

www.rsc.org/journalname

Despite decades of research, wound complications remain a major cause of postoperative mortality, especially in the face of multiple comorbidities. Addressing the issue of anastomotic leakages and impaired wound healing from a new angle is of great interest with the prospect of having direct impact on patient outcome. Recently, aqueous suspensions of silica and iron oxide nanoparticles have been employed to connect biological tissue by serving as an adhesive layer eventually leading to macroscopic gluing of tissue. In this work, we explore the prospects of this effect by introducing bioactive tissue adhesives composed of nanoparticles produced via scalable and sterile flame spray pyrolysis. We investigate six different metal oxides on cytocompatibility, hemostatic activity and adhesive properties in a small intestine lap joint model. While bioglass nanoparticles show exceptionally strong procoagulant and adhesive properties, the cell membrane integrity is impaired at high particle concentrations. Interestingly, when bioglass is combined with ceria, a material that has well-documented cytoprotective effects, the resulting hybrid particles exhibit the same beneficiary effects as bioglass while featuring superior cytocompatibility. Taken together, we demonstrate highly modular synthesis of nanoparticles expressing adhesive properties in conjunction with tailored bioactivity. Such bioactive nanoparticles as adhesion nuclei in wound healing have a wide range of potential applications in surgical wound care and regenerative medicine.

1 Introduction

Haemorrhage has been found to be the main cause of death within the first 24 hours of a traumatic injury¹. Emergency medical services and surgeons are challenged by poorly healing wounds as well as by bleedings that are difficult to staunch². The impeded barrier function of wounded tissue is also responsible for a comparatively high rate of post-operative complications in surgical patients. Iatrogenic injuries, anastomatic leakages and infections after resections³ or abdominal surgery^{4,5} pose inextricable problems to surgeons.

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Conventional anastomotic techniques such as suturing, stapling or taping are often insufficient for the management of abdominal wounds and anastomoses, especially on the long term. Recent studies have found that around 10 % of patients who undergo colorectal surgery suffer from subsequent anastomotic leakages⁴. Such leakages have a notoriously high morbidity (around 20 %) which illustrates how important a new wound management solution is. Furthermore, conventional anastomotic techniques do not offer active promotion of the healing process⁶. In recent years, a plethora of novel wound management solutions have addressed the shortcomings of conventional techniques. A range of biomaterials has been developed to figure as hemostats, adhesives, and sealants, especially for post-operative wound treatment^{7,8}. Natural materials used for these purposes, such as fibrin glues, have documented compatibility issues and lead to allergic reactions in some patients⁹. Synthetic surgical materials are more readily available, yet less adhesive and effective than their natural counterparts¹⁰.

Nanotechnology has given rise to a whole new tool set for the design of clinical biomaterials. Rose *et al.*¹¹ have shown that aqueous suspensions of silica nanoparticles glue biological tissue

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together by a mere physical phenomenon: The particles form an adhesive layer due to the adsorption of proteins onto their surface. The resulting bridging layer can sustain high amounts of stress due to protein rearrangement processes on the particle surface. While advantages compared to current wound closing techniques have been shown by Meddahi-Pellé *et al.*¹², the particles were solely tested on their adhesive properties, neglecting possible bioactivity and compatibility. Additionally, large quantities of high-quality nanoparticles are required in order to provide sufficient surface area for protein binding, posing a synthetic challenge to nanoparticle manufacturing. Nevertheless, the reported adhesive properties of nanoparticle suspensions are very promising and serve as a base for exciting research; a multitude of materials, architectures and applications are left to explore.

Here, we develop bioactive metal oxide nanoparticle-based tissue adhesives for wound management applications. We employ highly scalable liquid-feed flame spray pyrolysis (LF-FSP) to produce different metal oxide nanoparticles with comparable size and surface area from low cost chemical precursor solutions at full stoichiometric control. This one-step combustion process¹³ allows the reproducible synthesis of high quality¹⁴ metal oxide nanoparticles on an industrial scale up to kg/h^{15–18} without the formation of harmful byproducts and in absence of endotoxins. Furthermore, the freedom of choice in materials and the process modularity enables the design of oxide particles featuring complex architectures including hermetic coatings^{19,20}, Janus-shaped hybrid particles^{21,22}, and a variety of primary particle shapes²³. In order to have a meaningful set of nanoparticles, we selected silica and iron oxide particles for their documented ability to glue soft tissue¹² and bioactive metal oxide particles (45S5 bioglass, 13-93B3 borate glass, and ceria) for their beneficial effects in soft tissue healing²⁴. We systematically investigate these metal oxide particles on their suitability as tissue glues by first assessing their hemostatic and cytotoxic activities. We then demonstrate their ability to glue biological tissue in a small intestine model and benchmark the particles against commercial alternatives. Finally, we present the development of a hybrid particle that unifies the benefits of different materials.

2 Materials and methods

2.1 Particle synthesis

All non-commercially available particles were produced by liquid-feed flame spray pyrolysis (LF-FSP)¹³. 5 mL/min of appropriate precursor solutions was fed to a water-cooled spray nozzle, dispersed by 5 L/min O₂ with a pressure drop of approximately 1.5 bar at the nozzle tip. The spray was ignited by a premixed CH₄ / O₂ (1.5 L/min / 3.2 L/min) flame. Particles were collected on a glass fiber filter mounted above the flame.

The precursors were dissolved in equal parts of 2-ethylhexanoic acid and acetonitrile such that the total metal ion concentration of the solution was 0.3 mol/L. The precursor composition of the particles is as follows: SiO₂ (100 wt% HMDSO), Fe₂O₃ (100 wt% Iron(III) acetylacetonate), CeO₂ (100 wt% Cerium(III) nitrate), bioglass (41 wt% calcium 2-ethylhexanoate, 38 wt% sodium 2-ethylhexanoate, 3 wt% tributyl phosphate, 18 wt% HMDSO), bo-

rate glass (65 wt% tributyl borate, 16 wt% calcium acetylacetonate, 6 wt% sodium 2-ethylhexanoate, 1 wt% tributyl phosphate, 5 wt% magnesium acetylacetonate, 7 wt% potassium acetylacetonate).

Hybrid bioglass/ceria particles were produced by a modified FSP setup, similar to the one described previously by Starsich *et al.*²⁵. Ceria precursor solution (0.3 mol/L, composition as above) was fed to a water-cooled spray nozzle at a rate of 6 mL/min. It was dispersed by 5 mL/min O₂ with a pressure drop of approximately 1.5 bar at the nozzle tip. The spray was ignited by a premixed CH₄ / O₂ (1.25 L/min / 2.5 L/min) flamelet. The entire flame was enclosed in a stainless steel tube and sheathed with O₂ (20 L/min) and pressurized air (100 L/min). The bioglass precursor solution (0.2 mol/L, composition as above) was fed at 3 mL/min through a tube opening 11 cm downstream of the FSP nozzle and dispersed with 13 L/min of N₂ into the FSP plume²⁶. Particles were collected on a glass fibre filter mounted above the flame.

2.2 Particle Characterization

For all experiments, the produced nanoparticles were dispersed without addition of stabilizers. Aqueous suspensions of metal oxide particles are comparatively stable for hours²⁷ (see also ESI, Figure S1).

2.2.1 Transmission electron micrographs

Nanoparticles were suspended in 0.5 mL deionized water and a drop of 10 µL was placed on a clean parafilm surface. A grid with a formvar film (Electron Microscopy Science, Lucerna-Chem AG, Lucerne, Switzerland) was placed on top of the drop for 10 min. Any excess liquid was removed and the grid air-dried. After drying, the grid was imaged in a JEOL 2000FX transmission electron microscope (TEM).

2.2.2 Elemental analysis

Elemental analysis by inductively coupled plasma atomic emission spectroscopy (ICP-OES) was conducted by Bachema AG, Rütistrasse 22, CH-8952 Schlieren.

2.2.3 XRD measurements

X-ray diffraction patterns (XRD) were obtained with a Bruker D8 advance diffractometer (40 kV, 40 mA, CuKα radiation) at 2θ = 10°–70°.

2.2.4 Brunauer-Emmett-Teller absorption

The particle specific surface area (SSA) was measured by N₂ adsorption at 77 K using a five-point Brunauer-Emmet-Teller method (Tristar, Micromeritics) after degassing the samples for 1 h at 150 °C in N₂.

The density ρ and the specific surface area SSA was used to estimate the primary particle diameter d_{BET} using Equation 1²⁸.

$$d_{BET}[nm] = \frac{6000}{SSA[m^2/g] \cdot \rho[g/cm^3]} \quad (1)$$

2.2.5 Hydrodynamic diameter determination by dynamic light scattering

Particle suspensions of 0.13 mg/mL in ethanol were probe-sonicated for 5 minutes to break possible agglomerates. Three dynamic light scattering (DLS) measurements per sample were conducted using a Zetasizer (Nano ZS90, Malvern Instruments). In between measurements, the suspensions were vortex-mixed to ensure homogeneity. The hydrodynamic diameter d_{DLS} and polydispersity index (PDI) found for particle suspensions in distilled water can be found in Table S1 of the ESI.

2.2.6 Zeta potential measurements

Particle suspensions of 0.05 mg/mL in ultrapure water were sonicated in an ultrasonic bath for 5 min. Zeta potential measurements with automated pH adjustment were performed with a Zetasizer (Nano ZS, Malvern Instruments). Borate glass proved to be unstable in water, which rendered zeta potential measurements unreliable.

2.3 Assessment of hemostatic properties

The hemostatic properties of the produced nanoparticles were investigated based on the tiered framework for assessing nanoparticle biocompatibility developed by the National Cancer Institute's Nanotechnology Characterization Laboratory (NCI-NCL)²⁹. For comparison, the commercially available Aerosil 380 fumed silica particles (Sigma-Aldrich) were included in the set of particles. The nanoparticles were suspended in PBS according to their specific surface area such that the total particle surface was the same for all suspensions and equivalent to a silica nanoparticle concentration of 10 mg/mL (dry weight). Additionally 10-fold and 100-fold dilutions were created. All suspensions were vortex-mixed and left in an ultrasonic bath for 20 min. **To effectively remove particles from cellular suspensions before absorbance measurements in order to prevent interference, the suspensions were centrifuged at 6000 x g for 5 min as illustrated on Figures S5(a) and S5(b).**

A one-way ANOVA followed by a Bonferroni post-hoc test was performed to find statistical significance between metal oxide suspension performance in the hemolysis and cytotoxicity assays (GraphPad Prism version 6.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com).

2.3.1 Hemolysis

After obtaining written informed consent, whole blood from healthy volunteers was collected in tubes containing 0.109 mol/L citrate as an anti-coagulant and centrifuged at 800 x g. The plasma was collected for the coagulation measurements. The remaining red blood cells were resuspended in PBS and washed three times. The hemoglobin concentration was adjusted to 30 mg/mL. A 1 % Triton X solution was used as positive and PBS as negative control. 180 μ L red blood cells and 20 μ L of nanoparticle suspension were added to each well of a 96-well plate. After incubation for 3 h at 37 °C, the contents of the wells were extracted and centrifuged at 6000 x g for 5 min. 100 μ L of each supernatant was transferred to a well on a 96-well flat-bottom plate. Subsequently, the absorption at 570 nm (hemoglobin) was

measured with a Mithras² LB 943 multimode microplate reader.

2.3.2 Cytotoxicity

Monocytes (THP-1, human monocytic cell line) were cultured in RPMI-1640 supplemented with 10 % fetal bovine serum (FBS). For experiments, THP-1 cells were suspended in medium with 1 % FBS at a cell concentration of 5×10^5 cells/mL. 180 μ L of the THP-1 suspension and 20 μ L nanoparticle suspension were added to each well of a 96-well plate. After overnight incubation at 37 °C, the contents of the wells were extracted and centrifuged at 6000 x g for 5 min. 50 μ L of each supernatant was added to a well on a 96-well plate and combined with 50 μ L of CytoTox 96 reagent (CytoTox 96 Non-Radioactive Cytotoxicity Assay, Promega, Madison, WI USA). After 30 min incubation at room temperature, the absorption at 490 nm was measured with a plate reader (Mithras² LB 943).

2.3.3 Coagulation

20 μ L of nanoparticle suspension were added to tubes containing 180 μ L blood plasma each. After 30 min incubation at 37 °C, the contents of the wells were extracted and centrifuged at 6000 x g for 5 min. 100 μ L of each supernatant was transferred to a well on a 96-well flat-bottom plate and recalcified with 65 μ L of 50 mM CaCl_2 solution. Subsequently, fibrin polymerization was evaluated by measuring absorption at 405 nm in 50 s intervals during 1 h with a plate reader (Mithras² LB 943)³⁰. The clotting time is a measure for how long it takes a coagulation process to reach saturation. To evaluate this, the maximum absorbance of the wells containing plasma treated with Aerosil 380 was determined. The clotting time was defined as the moment a well reached an absorbance within 5 % of that value.

2.4 Adhesion on small intestine

Small intestine from pig (obtained from Schlachtbetrieb St. Gallen AG, Bazenheid, Switzerland) was stretched and cut to pieces of 2.5 cm width and 15 cm length with a scalpel. As a reference, Ludox TM-50 (Sigma-Aldrich), a commercially available aqueous suspension of silica nanoparticles, was included in the particle set. The pieces were cut in the middle and glued serosa to serosa by spreading 20 μ L of 10 wt% nanoparticle suspension between them. The overlap joint was 1 cm long and was pressed together for 1 min with a 2 kg weight. Subsequently, slips of paper were applied onto both ends of the glued piece to prevent sliding during the measurement. Tensile tests were performed on a Zwick Z100 machine at a pulling rate of 15 mm/s. The intestine was immersed in PBS until use and processed without any pre-treatment such as drying. During each measurement, the intestine was exposed to air during 10 min. To benchmark the particles against an existing solution, the fibrin sealant patch TachoSil (Baxter) was applied. TachoSil was pre-moistened in PBS and then used to bridge the two intestine pieces with the active (yellow) side. In a second adhesion measurement illustrated in **Figure 5(d)**, the pieces were cut to 3.5 cm width and 12 cm length.

SPSS (IBM SPSS, Chicago, IL, USA) was used for statistical analyses. Linear regression analysis was performed to assess the influence of metal oxide suspensions (independent variables) on

adhesion forces (dependent variable).

3 Results and discussion

3.1 Physicochemical characterization

LF-FSP allows the production of metal oxide particles with uniform size distribution (typically 20 to 200 nm primary particle size). Here, we produced five different metal oxide nanopowders and recorded transmission electron micrographs (TEM) of them. Figure 1(a) shows mostly spherical primary particles with comparable diameters below 100 nm. The CeO₂ particles feature smaller primary particle sizes than the other metal oxides. The borate glass particles exhibit networking between each other due to their reactivity in aqueous environments³¹.

The X-ray diffraction patterns in Figure 1(b) show that borate glass, bioglass, and SiO₂ are mostly amorphous with partial crystalline phases, while ceria and iron oxide are primarily crystalline. In agreement with Strobel and Pratsinis³², maghemite (γ -Fe₂O₃) is the predominant crystalline phase when iron oxide is produced in an open flame setting. The TEM images and XRD patterns of the bioglass nanoparticles are in good agreement with the original report on flame-made bioglass by Brunner *et al.*³³. Also the other produced metal oxide nanoparticles show morphologies and crystallinities comparable to previous reports on iron oxide¹⁹, borate glass³⁴, silica³⁵, and ceria¹³.

According to elemental analysis by ICP-OES, the produced bioglass nanoparticles are composed of 53 % SiO₂, 4 % P₂O₅, 18 % CaO and 25 % Na₂O. These ratios are in agreement with the theoretical composition of 45S5 bioglass (45 % SiO₂, 6 % P₂O₅, 24.5 % CaO and 24.5 % Na₂O).

The specific surface areas (SSA) of as-prepared nanoparticles determined by the Brunauer-Emmett-Teller (BET) method are shown in Table 1. All particles feature comparable specific surface areas ranging from 60 m²/g to 227 m²/g, with SiO₂ nanoparticles exhibiting the biggest SSA. The SSA equivalent average primary particle diameters (d_{BET}) displayed in Table 1 are in good agreement with the TEM images. Dynamic light scattering (DLS) measurements further show that all particles have comparable hydrodynamic diameters. Hydrodynamic size results shown here were determined in ethanol since DLS measurements of bioglass in water (see ESI, Table S1) showed high variation. In summary, the similarity in size, surface area and shape allows the comparison of the produced particles with respect to other properties, such as hemostatic and adhesive activities, both being critical for wound healing applications.

Table 1 BET and DLS analysis results. Mean values $\pm$ SD are reported.

Nanoparticle	SSA [m ² /g]	d_{BET} [nm]	d_{DLS} [nm]	PDI
SiO ₂	227 $\pm$ 3	10	176 $\pm$ 4	0.18
Fe ₂ O ₃	59.6 $\pm$ 0.2	19.2	165.3 $\pm$ 0.2	0.35
CeO ₂	84.5 $\pm$ 0.6	9.3	399 $\pm$ 4	0.31
Bioglass	110 $\pm$ 1	20	229 $\pm$ 4	0.23
Borate glass	68.1 $\pm$ 0.3	33.8	210 $\pm$ 10	0.35

Fig. 1 (a) Transmission electron micrographs of all produced metal oxide nanoparticles show comparable primary particle sizes. (b) X-ray diffraction patterns (XRD) of all as-prepared metal oxide nanoparticles reveal that silica, bioglass and borate glass are mostly amorphous, while maghemite and ceria are rather crystalline.

3.2 Hemostatic activity of flame made-metal oxide nanoparticles

When injury occurs, bleeding needs to be halted promptly to prevent excessive blood loss that otherwise could have detrimental consequences. To assess if and by how much the nanoparticles increase the coagulation rate, fibrin polymerization was measured over time in a plasma coagulation assay. For comparison, commercially available fumed silica nanoparticles with well-documented hemolytic³⁶ and procoagulant³⁰ activity (Aerosil) were included in the experiments. The native properties of the particles, especially surface charge, are pivotal on how proteins adsorb in biological environments. In turn, protein adsorption and protein corona formation are key processes that govern hemostatic and adhesive properties of the nanoparticle suspensions³⁷. Since protein adsorption prior to particle application will mask potential differences between different metal oxide particles³⁸, we characterized and applied native particle suspensions.

All particles except borate glass showed a significant increase in blood clotting speed as shown in Figure 2(a). Bioglass showed extraordinary procoagulant properties and saturation was reached substantially faster than other metal oxides particles. More specifically, saturation in plasma clotting was reached three times faster

Fig. 2 (a) Coagulation increase compared to PBS over time. Bioglass reaches saturation significantly faster than any other particle. $n=3$ independent samples per particle type, measured at a silica nanoparticle concentration of 0.1 mg/mL and surface-equivalent amounts of the five other particles. (b) Zeta potential of the particles at different pH values. All potentials are negative at physiological pH. $n=3$ measurements per pH. The mean values and SD are displayed. The coagulation speeds coincide with the negativity of the zeta potential at physiological pH.

in samples exposed to bioglass compared to other nanoparticles with well-documented procoagulant properties such as Aerosil.

As can be seen in Figure 2(b), the zeta potential at pH 7 is most negative for bioglass followed by silica, iron oxide and ceria. The order coincides with the respective coagulation speeds. This correlation is in agreement with Ostomel *et al.*³⁹ who showed that more negatively charged metal oxides triggered blood coagulation faster and accelerated the process more than neutral or positively charged particles. The reason for this is most likely the increased contact activation of coagulation factors at the particle-plasma interface which leads to higher clotting speeds⁴⁰.

3.3 Adhesion on soft tissue

Apart from coagulation, hemostasis can be reached by mechanically closing a wound. To be applicable for tissue gluing, the nanoparticles need to exhibit strong adhesive properties. Adhesion is not only necessary for gluing, but also to keep the particles localized on a wounded site. Some of the most promising potential uses of such nanoparticle-based tissue adhesives are topical applications to organs with heavy blood flow, such as parenchymal injuries of abdominal organs, and sealing of leakages of in-

testinal fluids. Since intestinal anastomotic leakages are among the most dreaded complications after abdominal surgery, the use of particle-based adhesives on intestine is of particular clinical relevance^{4,41}.

To quantify the adhesion on soft tissue, particle suspensions were spread between pieces of small intestine, as illustrated in Figure 3(a). Tensile force measurements on the glued pieces, shown in Figure 3(b), allowed quantification of the adhesive properties. After spreading the particles, the glued pieces were easily handleable and stable. The forces the pieces were able to withstand while being pulled apart vertically were measured and plotted as a function of the pulling distance. As an example, the force curves for bioglass are shown in Figure 3(c). Their shape indicates a rupture event at the peak of each curve, where the measured force had been building up until the piece ripped at the glued site. A comparison of all metal oxide particle suspensions is found in Figure 3(d). All particle suspensions exhibited measurable adhesion above 100 mN. Bioglass significantly improved adhesion ($\beta=0.679$, $R^2=0.461$, $p=0.015$) while other metal oxides had no significant influence. The bioglass-treated pieces were able to withstand forces of around 200 mN, which can be attributed to the well-documented strong tissue-bonding properties of bioglass²⁴.

Fig. 3 (a) The nanoparticles were spread on pieces of small intestine to glue them together. (b) The maximum tensile stress they could withstand was measured in a lap joint experiment. (c) Normalized force-distance curves for the lap joints glued with bioglass. The shape of the curves is typical for a rupture event. (d) Maximum tensile force values for all particles displayed in boxplots (whiskers show the minimum and maximum value). The gluing properties of bioglass surpass those of all other tested particles. $n=3$ independent samples per particle type.

3.4 Cytocompatibility

In addition to strong hemostatic and adhesive properties, it is pivotal that nanoparticle-based tissue glues are innocuous to cells. There is a high demand of oxygen in the microenvironment surrounding a wound due to the increased oxygen consumption by metabolically active cells. A deficiency of oxygen supply results

in impaired wound healing, which makes it especially important that applied nanoparticles do not harm erythrocytes⁴². Similarly, the cells involved in the inflammatory cascade are of crucial importance in the early stages of the wound healing process. Monocytes and macrophages play a big role in regulating the immune response to foreign bodies, cleaning the wound from debris and preventing infections⁴³. It has been shown that the toxicity of metal oxide particles is cell-type dependent and that surface charge and particle morphology govern cellular toxicity⁴⁴. Therefore, dose-dependent effects on the integrity of both human erythrocytes and monocytes were measured for the different metal oxide particles.

The hemolytic activity of the nanoparticles was assessed by incubation with human erythrocytes and measurement of freed hemoglobin. Similarly, the release of lactate dehydrogenase (LDH) was measured after 24 hours exposure of human monocytes to metal oxide nanoparticles. LDH is released into media from damaged cells and is a sensitive marker for cellular damage⁴⁵. As displayed in Figure 4, both cytotoxicity experiments demonstrate the substantially high lytic activity of silica nanoparticles, even at concentrations as low as 0.01 mg/mL ($\gg 50\%$ relative to full lysis by Triton X). This is in line with previous reports on the highly hemolytic activity of various silica nanoparticles attributing their toxicity to particle characteristics including geometry, porosity and surface charge⁴⁶. This finding also confirms that the application of highly concentrated silica nanoparticle suspensions poses issues concerning toxicity and biodegradability^{47–50}. Interestingly, surface-equivalent amounts of bioglass are less lytic and only show significant lysis at concentrations of 1 mg/mL. These results confirm earlier reports by Mačković *et al.* demonstrating high cytocompatibility of flame-made bioglass for concentrations of at least 200 $\mu\text{g/mL}$ ⁵¹. The fact that ceria particles show lower lysis than the PBS negative control and the amount of lysed cells decreases at increased concentration may be explained by their well-documented antioxidant properties⁵². A similar negative dose response has been observed for iron oxide nanoparticles. While the effect is present very consistently in both cytotoxicity assays and cannot be easily explained, trypan blue showed comparable viability for all iron oxide concentrations investigated (see Figure S5). Overall, a good correlation between hemolysis and monocyte viability was found for all particles on a similar dosage basis. Interestingly, the differences in zeta potential only partially account for the different cytolytic activities of the different metal oxide nanoparticles. While the zeta potential of silica and bioglass nanoparticles at neutral pH is comparable, silica is significantly more cytolytic. This corroborates earlier findings that cytolytic activity is governed not solely by surface charge, but also other factors, such as morphology and shape⁴⁴. Iron oxide and ceria on the other hand show comparable and less negative zeta potential values, and lower cytotoxicity than silica and bioglass.

3.5 Bioglass/ceria hybrid nanoparticle

In summary, it is evident that rapid treatment of a wound with bioglass nanoparticles could critically promote hemostasis and tis-

Fig. 4 (a) Hemolytic and (b) cytotoxic activity of the particles relative to Triton X (100 % lysis). All silica-containing particles show dose-dependent cell lysis. Ceria and iron oxide stabilize the cells. $n=3$ independent samples per particle type, measured at silica nanoparticle concentrations of 0.01 mg/mL, 0.1 mg/mL and 1 mg/mL, and surface-equivalent amounts of the other particles. The mean values and SD are displayed.

sue adhesion. Even though silica nanoparticles also show significantly increased coagulation, their inferior cytocompatibility^{47,50} makes them unsuited for direct application to a wound. When applied at high concentrations, however, bioglass also exhibits potentially unfavorable cytolytic activities as seen in Figure 4. In contrast, ceria nanoparticles have shown beneficial effects on cell viability but very weak procoagulant and adhesive properties as indicated in Figure 2(a) and Figure 3. We therefore sought to unify both properties, i.e. high biocompatibility and strong adhesion, in a hybrid particle. By feeding more than one type of precursor to a flame spray reactor, hybrid nanoparticles can be produced with full stoichiometric control. To test the hypothesis that a hybrid particle could unite procoagulant, adhesive and cell-stabilizing properties, a bioglass/ceria nanoparticle (85 wt% CeO₂, 15 wt% bioglass) was produced by LF-FSP. Characterization of three different batches of said hybrid particles confirms good reproducibility of the synthesis process. Transmission electron micrographs and energy-dispersive X-ray spectra show that the hybrid particles consist of larger ceria crystallites (up to 50 nm) surrounded by fine bioglass particles (ESI, Figure S2). X-ray diffractograms (ESI, Figure S3) show characteristic ceria peaks masking the signal of the amorphous bioglass. The hydrodynamic diameter of the hybrid (440 ± 20 nm, PDI = 0.46) is compara-

ble to bioglass and ceria particles (see Table 1). Figure 5 offers a comparison of the hybrid particle to the single components. Figure 5(a) and Figure 5(b) show that the lytic effect of bioglass/ceria nanoparticles is significantly lower than bioglass only, both on human erythrocytes (0.1 mg/mL: $p < 0.05$) and monocytes (1 mg/mL: $p < 0.001$). This demonstrates that the addition of ceria in the nanoparticle has indeed reduced the lytic effect exhibited by bioglass, especially at concentrations of 1 mg/mL. The cytocompatibility experiments show that the bioglass character of the hybrid particles is relatively high, given that they only contain a small amount of bioglass. This can be explained by the fact that the hybrid particles consist of small bioglass particles located on the ceria surface. Thus there is a comparatively large bioglass surface area exposed despite its low content. Figure 5(c) indicates that the clotting time of the hybrid particles is comparable to that of bioglass and significantly shorter than those of other metal oxide particles. This again falls in line with the surface charge of the hybrid particles (ESI, Figure S4) which is close to bioglass, located between the zeta potential of pure ceria and bioglass. The short clotting time also confirms that the bioactivity of the hybrid is heavily influenced by the bioglass part.

Fig. 5 (a,b) The hemolytic and cytotoxic activity of the hybrid particles at high concentrations was significantly lower compared to bioglass. **(c)** The hybrid particles show similar procoagulant properties as bioglass, outperforming all other particles. $n=3$ independent samples per particle type. The mean values and SD are displayed. **(d)** Tensile force measurements show highly adhesive properties for the hybrid particle, surpassing those of other particles and TachoSil. $n=3$ samples per particle type. Whiskers show the minimum and maximum value.

In order to better understand how the measured adhesion of the metal oxide nanoparticle formulations compares to alternatives, we benchmarked the bioglass/ceria nanoglue against Ludox, iron oxide and TachoSil. Ludox is a commercially available silica nanoparticle dispersion and has shown strong adhesive properties¹¹. Similarly, iron oxide nanoparticles have demonstrated strong adhesion to skin and liver tissue¹². TachoSil is a fibrin sealant patch that is widely used to close and seal blood vessels after surgery⁵³ and has been praised for its high adhesive

strength on soft tissue⁵⁴. The tensile force measurement results are displayed in Figure 5(d). Comparison with the adhesion experiment displayed in Figure 3 shows similar forces for iron oxide treated tissue, despite different widths of the overlap joints (2.5 vs 3.5 cm). This can be explained by the fact that the tissue gluing properties of the nanoparticle suspensions not containing bioglass are comparatively low in this setting and therefore differences in force are not easily detectable between different sizes of glued areas. The hybrid particles significantly improved tissue adhesion ($\beta=0.873$, $R^2=0.762$, $p<0.001$). No significant influence was found for iron oxide particle suspensions, Ludox 10% and TachoSil.

Taken together, the hybrid particles exhibit extraordinary properties and successfully consolidate the advantages of bioglass and ceria. The addition of small quantities of bioglass to a nanoparticle results in strong adhesive and procoagulant properties. While showing the same increase in coagulation speed and strong adhesive properties as pure bioglass, the hybrid particles are less harmful to human erythrocytes and monocytes. These results demonstrate that particle characteristics can be tuned selectively by adequate changes to the synthesis conditions, such as precursor composition and reactor settings. This freedom allows tailoring particle properties to fit the need of certain prospective applications, such as treating postsurgical abdominal injuries.

With regard to clinical translation of metal oxide based nanoglues, compatibility and degradability take center stage. Numerous *in vivo* and *in vitro* studies have shown that both ceria^{55,56} and bioglass^{51,57} nanoparticles are well tolerated. Furthermore, they exhibit biologically relevant activities that make them suitable for a range of medical applications. Bioglass promotes the growth of new tissue and features strong adhesion to soft tissue²⁴. It has been used in the clinics for more than three decades⁵⁸, mostly to repair or replace damaged parts of the musculo-skeletal system⁵⁹. In recent years, the potential of bioglass for soft tissue repair has become evident and several studies have reported compelling results concerning wound healing and the promotion of angiogenesis using bioglass-based fillers^{24,60,61}. Since ceria figures as a reactive oxygen species (ROS) scavenger, it exhibits several exciting properties which include improved wound healing^{62–64}, anti-inflammatory⁵² and anti-fibrotic⁶⁵ activity, and angiogenetic properties⁶⁶. Our results show that merging the benefits of these two clinically relevant materials is possible and in view of their additional bioactive properties, they offer great prospects for comprehensive wound healing solutions.

4 Conclusions

In this work we produced several metal oxide nanoparticles by flame spray pyrolysis and investigated their hemostatic and adhesive properties. The bioactive ceramic bioglass promotes coagulation to a great extent and features remarkable adhesive properties, outperforming other metal oxide nanoparticles such as silica, iron oxide or ceria in both fields. We demonstrated that a bioglass/ceria hybrid nanoparticle exhibits the same procoagulant and adhesive properties as bioglass while being significantly less cytolytic. These results illustrate that particle properties can be

devised by choosing the appropriate materials and that more sophisticated particles could exhibit even more exciting properties. Due to its versatility, there are many potential medical applications of this nanoparticle based approach. While there are various solutions on the market, this non-invasive and effective method is a complement to existing ones. Before clinical application, more investigation on biocompatibility is necessary.

The nanoglue developed here unifies gluing properties with bioactivity in a relatively simple formulation of constituents with well-documented biological compatibility. Introducing bioactive nanoparticles as adhesion nuclei enables engineering the microenvironment of a wound in support of the natural repair process. Importantly, recent progress in tissue engineering stipulates novel solutions for attaching scaffolds to biological tissues in a minimally invasive way that promotes integration. Hence the developed nanoglue could, in addition to all the obvious applications, find uses well beyond classical wound healing.

5 Acknowledgements

We thank Ursina Tobler, Yvonne Elbs-Glatz and Olivia Neururer for technical support. This research was partially funded by the Swiss National Science Foundation (grant no. 205320_163243).

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