Bisphosphonate nanoclay edge-site interactions facilitate hydrogel self-assembly and sustained growth factor localization

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Nanoclays have generated interest in biomaterial design for their ability to enhance the mechanics of polymeric materials and impart biological function. As well as their utility as physical cross-linkers, clays have been explored for sustained localization of biomolecules to promote in vivo tissue regeneration. To date, both biomolecule-clay and polymer-clay nanocomposite strategies have utilised the negatively charged clay particle surface. As such, biomolecule-clay and polymer-clay interactions are set in competition, potentially limiting the functional enhancements achieved. Here, we apply specific bisphosphonate interactions with the positively charged clay particle edge to develop self-assembling hydrogels and functionalized clay nanoparticles with preserved surface exchange capacity. Low concentrations of nanoclay are applied to cross-link hyaluronic acid polymers derivatised with a pendant bisphosphonate to generate hydrogels with enhanced mechanical properties and preserved protein binding able to sustain, for over six weeks in vivo, the localized activity of the clinically licensed growth factor BMP-2.
issue engineering and regenerative medicine seeks to harness the developmental potential of stem cells to replace tissue lost or damaged through injury or disease. Biomaterials such as hydrogels serve in this context to provide a 3D template, or scaffold, able to promote new tissue growth through provision of environmental cues that direct stem cell function. Controlled, highly localized biomolecule presentation in particular, is key for coordinating cell migration, proliferation, and differentiation. To date, the traditional drug delivery paradigm, is key for coordinating cell migration, proliferation, and differentiation. To date, the traditional drug delivery paradigm of slow release has dominated attempts to control the presentation of biomolecules by hydrogel scaffolds. However, slow release strategies are typically poorly suited to achieving the precisely localized signaling mechanisms at play in natural morphogenesis and present challenges for promoting tissue ingress into spaces occupied by the drug-releasing hydrogel.

Nanoclays offer new opportunities for biomaterial design. In particular, nanoclay–protein complexes allow localized activity of bioactive molecules within a hydrogel environment permissive for cellular ingress. This approach has been applied to achieve high loading and localized delivery of insulin-like growth factor-1 mimetic protein, localization of vascular endothelial growth factor to initiate the formation of new blood vessels at an injury site, and localization of bone morphogenetic protein (BMP)-2 to achieve ectopic bone formation at the lowest dose recorded in the literature to date. Furthermore, the interaction of nanoclays with polymers is also of core interest in biomaterial design for their utility as physical cross-linkers that combine the dynamic properties associated with physical hydrogels such as self-assembly and self-healing with greatly enhanced mechanical stiffness and toughness.

Despite the clear utility of clay–polymer and clay–protein interactions for biomaterial design, the ability to exploit both interactions, simultaneously, has proved challenging. For example, where clay–protein interactions have been applied to modify

Fig. 1 Laponite bisphosphonate interactions allow physical cross-linking of hydrogels. Laponite addition (2% wt. vol.) to a bisphosphonate (BP) functionalized (DS = 25%) hyaluronic acid (HA) polymer (2% wt. vol.) yields a stiff gel, while no gel is formed following addition of Laponite to HA alone (a graph displays storage ($G'$) and loss ($G''$) moduli of gels under a frequency sweep). HA-BP-Laponite gels can be manipulated using forceps and self-heal (b). As well as achieving Laponite cross-linking via BP disulfide functionalization of the HA backbone (HA-SS-BP; c and d), thiol functionalization of the Laponite particles themselves via incubation with low molecular weight thiol-terminated BP derivatives (BP-SH) was also achieved (f). Thiol functionality of Laponite-BP-SH was confirmed via successful gelation of hyaluronic acid modified with dithiopyridyl groups (HA-SSPy) following addition of Laponite-BP-SH, but not of BP-SH derivatives alone (e). Thiol functionality of Laponite-BP-SH was also confirmed via successful photoinduced gelation of allyl-functionalized HA in the presence of Laponite-BP-SH. No gelation of HA-allyl + Laponite-BP-SH was observed in the absence of UV exposure (g). Treatment of the formed gels with dithiobisethanol (DTT) resulted in dissolution only of those nanocomposite gels where BP groups were conjugated via a labile disulfide bond (h). $G'$ measurements were conducted at a frequency of 1 Hz.
release from a polymeric network, the formation of the clay–biomolecule complex dominates in the design process and minimizes the contribution of the nanoclay phase to the physical properties of the hydrogel. On the other hand, where polymer–clay nanocomposites have been optimized for their mechanical properties, any influence of clay on the drug release profile is often minimal and secondary to the clay’s primary influence on the polymeric network (e.g., through reduced swelling or degradation). This competitive compromise between strategies can be explained, at least in part, by the fact that both applications classically seek to employ the same site of interaction: the negatively charged surface of the clay particle. To our knowledge, strategies that utilize the clay edge-site for physical cross-linking of polymers, and the opportunities for material science this approach may afford, have not yet been explored.

LAPONITE® (Na,(Mg3-hLih)Si4O10(OH)2·nH2O), a synthetic smectite manufactured by BYK-ALTANA (herein, Laponite) consists of 25–30 nm diameter particles of 1 nm thickness, which possess a permanent negative surface charge and positive (amphoteric) charge in the form of Mg–OH (and some Si–OH) groups on the particle edge. Due to Laponite’s small particle diameter compared with natural smectites, the chemistry of the edge surface (representing ~7.4% of Laponite surface area versus ~0.2% of Montmorillonite) plays a particularly important role in determining the physical properties of Laponite colloids. For example, in the presence of ionic solutes, Laponite particles associate via edge–face interactions to generate gels, or, at high ionic strengths, flocculates. Tetravalent pyrophosphate salts are applied commercially to inhibit aggregation and improve dispersion by complexing with, and thus screening, the hydroxyl groups exposed at the particle edge.

Bisphosphonate (BP) is the organic analog of pyrophosphate. The additional valency of the carbon atom, which substitutes for the phosphate-bridging oxygen, provides possibilities for conjugation of BPs to functionalize polymers or other low molecular weight molecules. This approach has been applied, for example, to utilize the strong affinity of BPs for bone-bound calcium, facilitating targeting of BP-conjugated drugs or nanoparticles to bone surfaces. Ossipov et al. have extended this concept to generate in situ forming nanocomposite hydrogels by utilizing the coordination of tethered BP ligands with CaP nanoparticles to cross-link a hyaluronic acid (HA) polymer backbone. A similar self-assembly approach, applying the affinity of BP for magnesium, has recently been explored using assemblies of free
Gel storage modulus was dependent on nanoclay concentration and on the nature of the BP attachment chemistry (Supplementary Fig. 2). For example, a gel containing 2% Laponite displayed approximately double the storage modulus of a gel containing 1% Laponite (Supplementary Fig. 2a). Thiol-ene photochemical addition of BP-acrylamide to HA-thiol allowed attachment of approximately three BP groups to one thiol group of HA to produce a brush-like arrangement of BP groups along the HA backbone (termed HA-(BP)$_3$, degree of substitution (DS) = 15%)$^{18}$. This approach generated a gel with a 5x higher storage modulus following the addition of Laponite than gels generated through a disulfide attachment of BP groups to the HA backbone (HA-SS-BP, DS = 25%). The disulfide attachment, as well as being more labile than the acrylamide-thiol linkage, allowed the tethering of only one BP group per side chain. SEM analysis of physically cross-linked HA-(BP)$_3$ • Laponite hydrogels following freeze-drying revealed a nanoporous structure of fibrous aggregates which contrasted with the considerably more uniform appearance of freeze dried HABP chemical gels at the same submicron scale (Fig. 3).

To confirm the involvement of Laponite edge sites in the interaction with BP functional groups, Laponite nanoparticles were preincubated with pyrophosphate ions prior to mixing with HA-(BP)$_3$ (Fig. 4a, b). This reduced the stiffness of the gels by half ($G' = 2277$ Pa at 1 Hz), compared with gels prepared in the absence of pyrophosphate ($G' = 5660$ Pa at 1 Hz). This is consistent with competition for nanoclay Mg–OH edge sites. Gel stiffness partially recovered ($G' = 3638$ Pa) following swelling in PBS, indicating the release of pyrophosphate and the resultant formation of new physical cross-links. Notably, HA-BP • Laponite physical gels prepared in pure water were resistant to swelling (Fig. 4a). Preincubation of HA-(BP)$_3$, with BP-competing Mg$^{2+}$ ions did not result in an equivalent weakening, but rather achieved a further increase in gel stiffness ($G' = 6400$ Pa, Fig. 4c), likely due to the interlayer Mg$^{2+}$ cation bridging of BP-bound clay particles. Accordingly, the osmotic release of interlayer Mg$^{2+}$ upon swelling, caused an overall reduction in gel stiffness to below that observed for the gel prepared in pure water. This result reveals that swelling-resistant BP • Laponite cross-links were, in fact, compromised by addition of Mg$^{2+}$ consistent again with competition for bisphosphonate groups.

### Bisphosphonate functionalization of nanoclay edge-sites.

To explore the application of BP–Laponite interactions for nanoclary functionalization, thiolated low molecular weight BP molecules (BP-SH) were incubated with Laponite with the aim of imparting thiol-functionality to Laponite (Fig. 1c). Addition of Laponite • BP-SH to polymeric HA-SSPy derivative resulted in gel formation through a chemical thiol-disulfide exchange reaction, whereas addition of BP-SH without Laponite did not yield a gel. SEM analysis on the freeze dried gels again revealed a nanoporous structure of fibrous aggregates that is not apparent in chemically crosslinked HABP gels in the absence of clay. Interestingly this approach seemed to yield a more uniform pore distribution compared with the physically cross-linked HA-(BP)$_3$ • Laponite hydrogels, perhaps due to the improved miscibility of Laponite • BP-SH particles due to edge site OH screening (Fig. 3). To further prove the principle using a second chemistry, a gel was also formed through UV photo-initiated thiol-ene addition reaction of Laponite • BP-SH to allyl-derivatized HA (see structure in Fig. 1c(v)). Again, gelation did not occur in the absence of UV light, confirming the specificity of the reaction. Finally, treatment of the formed gels with dithiothreitol (DTT) resulted in dissolution of those nanocomposite gels where BP groups were conjugated via a labile disulfide bond, but not gels formed through photo-initiated thiol-ene cross-linking, as the thioester...
bond linking of Laponite•BP to HA is insensitive to DTT. Taken together, these results provide confirmation for the specificity of the clay–bisphosphonate interaction and open up exciting new opportunities for functionalizing Laponite edge sites.

**Protein binding by bisphosphonate-tethered nanoclay.** To explore the potential of BP bound Laponite for growth factor delivery, we examined protein retention and release in vitro and in vivo (Fig. 5). Hydrazine cross-linked HABP gels were prepared with and without Laponite and loaded with either a positively or negatively charged model protein (lysozyme, MW 14.4 kDa; pI 11.5) and bovine serum albumin, MW 66.5 kDa; pI 4.7, respectively). Consistent with the preserved availability of Laponite cation exchange sites, no release of loaded lysozyme was detected from HABP + Laponite gels over 7 days. HABP gels alone displayed binding affinity for lysozyme (as in other studies) and the release profile corresponded with HA leaching (in contrast to HABP + Laponite gels from which no release of either Laponite or HA was detected) indicating that an indirect influence of Laponite on hydrogel stability played a role in limiting release. Interestingly, and in contrast to its rapid release from HABP alone, HABP + Laponite gels also restricted the release of negatively charged albumin over 7 days. This is consistent with studies that describe patch binding onto Laponite surfaces of negatively charged proteins, including BSA, via adsorption onto clay of local acidic residues exposed on the protein surface.

The protein loading capacity of HA-BP•Laponite physical gels was also assessed (Supplementary Fig. 3). Following equilibration in a 10 mg ml⁻¹ solution of cytochrome C (Mw 11.7 kDa; pI 9.6) HA-BP•Laponite gels displayed a very high loading capacity (9.83 mg ml⁻¹) compared with HA and HABP hydrazone cross-linked analogs in the absence of clay (1.08 and 3.57 mg ml⁻¹, respectively). Again, negligible release of the loaded protein was observed from the HA-BP•Laponite gels over the subsequent 6 day incubation period (Supplementary Fig. 3b).

To test the ability of Laponite bound protein to promote a regenerative response in vivo, the above study design was repeated, substituting labeled lysozyme for a low concentration (5 µg ml⁻¹, 300 ng per gel) of the bone inductive growth factor, BMP-2. Consistent with previous studies, both HABP alone and Laponite alone (here, in contrast to previous studies, delivered as a low concentration sol) were able to achieve detectable bone induction with addition of low dose BMP-2, albeit variably and at low volumes (Fig. 6). In contrast, after 4 weeks, HABP + Laponite gels displayed strong and highly localized Cy7 dye intensity even 42 days (6 weeks) after implantation.

To assess the localization of loaded protein in vivo, Cy7-labeled lysozyme was loaded within hydrazine cross-linked HABP gels with and without Laponite (1%) and implanted subcutaneously in mice before scanning of fluorescence over 6 weeks (Fig. 5b). Unbound 1% Laponite solutions were implanted as a further control. Notably, while Cy7-labeled lysozyme was rapidly lost from HABP gels alone, HABP bound Laponite displayed strong and highly localized Cy7 dye intensity even 42 days (6 weeks) after implantation.

**Fig. 4 HA-BP•Laponite gels are compromised by edge-site or bisphosphonate-associating ions.** Compared with gels prepared in the absence of pyrophosphate (a), screening of Laponite edge sites with pyrophosphate ions (PPi) prior to mixing with HA-(BP)₃ reduced the stiffness of the gels by half. Stiffness partially recovered post swelling suggesting release of PPI and the formation of new physical cross-links (b). Preincubation of HA-(BP)₃ with BP-competing Mg²⁺ ions caused an initial increase in gel stiffness consistent with Mg²⁺ cation bridging of Laponite particles. Osmotic release of interlayer Mg²⁺ revealed that swelling-resistant BP•Laponite cross-links (a) were compromised by addition of Mg²⁺ (c). Storage modulus ($G'$) is recorded at 1 Hz.
harvest at 6 weeks, only HABP + Laponite gels remained recoverable from the implant site while HABP in the absence of Laponite had been completely resorbed. Histological analysis revealed that with addition of BMP-2, the hydrogel, largely preserved in the absence of BMP-2, appeared to be almost completely replaced by new bone tissue (Fig. 6). Thus after, 6 weeks in vivo, BP-bound Laponite was able to both stabilize HA hydrogels and bind, protect and sustain localized protein concentrations to enhance bone induction.

Discussion

In summary, we have demonstrated the potential to exploit bisphosphonate nanoclay edge–site interactions to generate a new class of nanocomposite with enhanced functionality through preserving interlayer or particle surface cation exchange sites (Fig. 7). Using this approach we have generated self-assembling, high water content, negatively charged polymer hydrogels that display dramatic enhancements in mechanical properties. We have also provided proof of concept for the use of low molecular weight bisphosphonated groups to impart edge–site functionality to clay nanoparticles. Furthermore, we have demonstrated the utility of bisphosphonate anchored Laponite to sustain the localization of active proteins in vivo over many weeks to achieve, as proof of concept, bone induction at a greatly reduced effective dose of BMP-2. This new approach to nanocomposite assembly has broad applications across healthcare and beyond.

Methods

Preparation of physical hydrogels. Physical hydrogels were prepared by interaction of HA derivatized with BP groups and Laponite nanoparticles. Two types of HA-BP derivatives were used in which BP groups were linked either through disulfide bonds (HA-SS-BP) or by thiol-ene addition to HA-SH derivative yielding multiple BP ligand attachments (HA-BP)24,25. Full methods for the preparation of HA derivatives are provided in Supplementary Information and Supplementary Fig. 6. Structural characterization of HA-SS-BP derivative was performed by 1H-NMR. DS with BP groups was calculated by comparative integration of HA acetamide peak and the ten methylene protons peaks23. For fabrication of hydrogels of 0.3 mL by volume, 150 μL of freshly prepared solution of Laponite at either 4% or 2% concentration was mixed with 150 μL of HA-BP derivative in water. The mixed solutions were transferred into 2 mL syringes, sealed with Para-film to prevent evaporation and kept overnight for gelation. The formed hydrogels of cylindrical shape (with 8 mm diameter) were removed from the syringe molds. HA concentration in the hydrogels was 2%, whereas Laponite concentration was 2% or 1%. Control experiments were also performed in which HA-BP derivatives were substituted with native HA. The influence of pyrophosphate ions on gelation was studied by preincubation of 8% Laponite solution with equal volume of 0.3 M Na2HPO4 × 10H2O solution for 1 h prior to the mixing with HA-BP derivative. Similarly, for the study of magnesium ions on gel properties, 6% MgCl2 (50 μL) prior to the mixing with Laponite solution.

Preparation of chemically cross-linked hydrogels. Hydrazone cross-linked HA hydrogels with or without Laponite were prepared by mixing hydrazide and aldehyde-modified HA derivatives. Four types of hydrogels were obtained: (1) HA hydrogel without attached BP groups (HA gel), (2) HA hydrogel without attached BP groups and with encapsulated Laponite NPs (HA + Laponite gel), (3) HA hydrogel with attached BP groups (HABP gel), (4) HA hydrogel with attached BP groups and with encapsulated Laponite (HABP + Laponite gel). Common aldehyde-modified HA derivative (HA-al) was used for all four types of the hydrogels. To obtain the hydrogels from groups 1 and 2, hydrazide-modified HA hydrogels of cylindrical shape (with 8 mm diameter) were removed from the syringe molds. HA concentration in the hydrogels was 2%, whereas Laponite concentration was 2% or 1%. Control experiments were also performed in which HA-BP derivatives were substituted with native HA. The influence of pyrophosphate ions on gelation was studied by preincubation of 8% Laponite solution with equal volume of 0.3 M Na2HPO4 × 10H2O solution for 1 h prior to the mixing with HA-BP derivative. Similarly, for the study of magnesium ions on gel properties, 6% MgCl2 (50 μL) prior to the mixing with Laponite solution.
A derivative (HA-hy) was used, whereas to obtain the hydrogels from groups 3 and 4, HA dually modified with hydrazide and BP groups (BP-HA-hy) was used. Firstly, solutions of Laponite and the HA components in water were prepared at 4% and 3%, respectively. The HA + Laponite and HABP + Laponite hydrogels of 0.3 mL volume were obtained by mixing of 25 μL of water with 75 μL of Laponite, 100 μL of HA-al, and 100 μL of hydrazide component (either HA-hy or BP-HA-hy) subsequently. For preparation of HA and HABP hydrogels, Laponite was substituted with water. The mixtures were quickly transferred into syringe molds to prevent evaporation and kept overnight, as described for the preparation of physical hydrogels.

Hydrogel characterization. Rheological characterizations of all hydrogels were performed using an AR2000 Advanced Rheometer (TA Instruments) with aluminum parallel plate geometry of 8 mm diameter. Frequency sweeps from 0.1 to 10 Hz were performed by monitoring storage ($G'$) and loss moduli ($G''$) at a fixed normal force (0.015 N) and a fixed strain. Frequency oscillation sweep from 0.1 to 10 Hz was performed by monitoring storage ($G'$) and loss moduli ($G''$) at fixed strain of 1%. Strain oscillation sweep from 0.2 to 100% was performed by monitoring storage ($G'$) and loss moduli ($G''$) at fixed frequency of 0.5 Hz. Time oscillation sweep was performed by applying strain at low (0.2%) and high (50%) values in an alternating manner but at the fixed frequency of 0.5 Hz. All experiments were repeated three times. Rheology plots represent measurements taken from the same sample. Mechanical properties were studied for as prepared hydrogels as well as after their equilibrium swelling in PBS for 24 h. For scanning electron microscopy (SEM) analysis hydrogels were freeze-dried and coated with 8–10 nm of Au/Pd before analysis on SEM (LEO 1550, Zeiss).

In vitro release of proteins and degradation of hydrogels. In vitro release of lysozyme (egg white) and albumin (bovine serum) from hydrazone cross-linked HABP hydrogels with or without Laponite and their degradation were evaluated.

**Fig. 6** Bisphosphonate-tethered Laponite sustains retention of functional BMP-2. Laponite, HABP, and HABP + Laponite were loaded with low doses (5 μg ml$^{-1}$) and implanted subcutaneously in mice to assess ectopic bone induction. False color μCT reconstructions of ectopic bone 6 weeks post implantation (a). HABP + Laponite delivered BMP-2 to induce significantly higher volumes of bone compared to either HABP or Laponite alone and sustained a significant increase in both bone volume (b) and density (c) over 6 weeks (error bars = standard deviation, n = 6; **, *** and **** indicate $P$ values of 0.01, 0.001, and 0.0001, respectively, two-way Anova, Tukey’s multiple comparisons test). Histological analysis of the recovered hydrogels revealed extensive regions of Sirius red stained bone tissue only in HABP + Laponite hydrogels incorporating BMP-2 (d). Auramine O staining (yellow) of Laponite indicates extensive degradation of the hydrogel structure with addition of BMP-2 (d). Scale bar = 50 μm.
where LOD were calculated for the standard curves of lysozyme, HA, and acid carbazole assay. Brieﬂy, 50 µl of the supernatants was placed in a 96-well plate and ﬂuorescence was measured at 475 nm excitation and 550 nm emission. All the concentrations of protein, HA, and Laponite were determined based on their standard curve in comparison with blanks. The lower limits of detection (LOD) were calculated for the standard curves of lysozyme, HA, and Laponite where release from HABP + Laponite gels was below the detection limits of the assay. LOD was calculated as follows:

\[
\text{LOD} = 3s / \sigma
\]

where \(s\) is the standard deviation of the intercept of the response (ﬂuorescence) and \(\sigma\) is the slope of the curve.

**In vivo release of lysozyme from hydrogels.** All animal studies were carried out following approval from the local Animal Welfare and Ethics Review Board (AWERB) University of Southampton and carried out in accordance with the guidelines and regulations stipulated in the Animals (Scientiﬁc Procedures) Act, UK 1986 under the approved Home Ofﬁce Project license (PPL 30/2880). Female MF1 wild type mice were anaesthetised with an intra-peritoneal injection of a mixture of hypnorm and hypnovel (1:3). 18 µl of HABP, Laponite, and HABP + Laponite hydrogels with 2 µl of Cy7 labeled lysozyme (Nanocs Inc., USA) or 2 µl of DW were placed in a 1 ml syringe mold. Totally, 2% HABP, 1% Laponite, and 2% HABP + 1% Laponite hydrogels with or without Cy7 labeled lysozyme were implanted into back subcutis (n = 6). Each mouse received three hydrogels on the left side and three hydrogels incorporating Cy7 labeled lysozyme. As a control, Cy7 labeled lysozyme solution was injected. To assess in vivo release of the labeled lysozyme, mice were scanned immediately after injection of the samples (initial lysozyme intensity) using an in vivo imaging system (IVIS, Perkin Elmer, Hopkinton, MA) at 660 nm (excitation) and 710 nm (emission) wavelengths. At each time point (3 days and 1, 2, 4, and 6 weeks), mice were scanned before sacriﬁce at week 6 and ﬁnal scanning (lysozyme intensity post implantation).

**In vivo ectopic bone formation.** Totally, 60 µl of 2% HABP, 1% Laponite, and 2% HABP + 1% Laponite hydrogels with or without BMP-2 (5 µg ml⁻¹) were placed in a 1 ml syringe mold before subcutaneous implantation as above (n = 6). At week 0, 2, 4, and 6, the mice were scanned using a microcomputed tomography (micro-CT, Skyscan 1176, Bruker, Kontich, Belgium) with 45 kV, 556 µA, 0.2 mm Al ﬁlter and a pixel size of 18 µm. Images were reconstructed using NRecon software with correction for misalignment and ring artefacts. To quantify the bone volume of each samples, the reconstructed images were analyzed using CTAn software. CTvox was implemented to create and visualize the 3D models of samples.

The samples were collected after sacriﬁce 6 weeks post implantation and were ﬁxed in 4% paraformaldehyde in PBS at 4°C for 3 days. Subsequently, the ﬁxed samples were embedded in parafﬁn and sectioned (7 µm). Acanth blue, Sirius red, and Auramine O staining was performed according to standard protocols. Images were captured with an inverted light microscope (Zeiss).
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**Author contributions**

Y.K. designed and carried out the in vitro and in vivo protein release and bone induction studies with assistance from S.L. for micro-CT analysis. D.O. carried out the hydrogel design, synthesis, and characterization with assistance from X.Y. and L.S. The original conception of the project and funding was through J.D., R.O., and J.H. The conceptualization of the nanocomposite strategy was by J.D and D.O. who, with Y.K. also wrote the paper with the assistance from all other coauthors. All authors have given approval to the final version of the paper.

**Competing interests**

The authors declare the following competing interests. The underlying technology has been patented and licensed to a University of Southampton spin-out company. Jonathan Dawson, Jons Hilborn, Dmitri Ossipov, and Richard Oreffo are shareholders in Renovos. The remaining authors declare no competing interests.

**Additional information**

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