Marine inspired biosilica-filled hydrogels

Sprawozdania

Informacje na temat projektu

DiaDOM

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Final Report Summary - DIADOM (Marine inspired biosilica-filled hydrogels)

This research combines two marine-inspired technologies to create transformative surgical glue for bone repair. Biomimetic approaches that mimic natural glues with synthetic materials, offer solutions more suitable to the body. In nature, glues underpin many biological systems, often performing their function in wet and turbulent environments, e.g. the blue mussel (Mytilus edulis). At present, strategies in bone-to-bone fixation mainly involve the use of mechanical fasteners (e.g. plates, nails, wires). Surgical glue could provide a simple and fast method to fix factures, particularly highly comminuted fractures where there are many small fragments that are difficult to fix using conventional fasteners. Currently, there are no clinically available bioadhesives that are suitable for hard tissue applications. This fellowship provided an opportunity to work with Prof Messersmith's Group, a world-leading expert in biomimetic adhesives systems who have successfully reverse engineered a synthetic bioadhesive that mimics the glue of the blue mussel (Mytilus edulis). While this glue has clinically proven soft tissue applications, in its current form further development is needed to develop its potential for hard tissue applications. The aim of this project was to improve the mechanical properties, injectability and osteogenic potential of this marine inspired bioadhesive for use in orthopaedics by adding marine silica fillers. The return phase was hosted at Queen's University Belfast, and involved a proof-of-concept animal study to test the silica filler used in our adhesive system.

The aim of the first phase of the project was to develop a protocol for combining the hydrogel system with biosilica (bioSi). This ceramic phase was added to improve the osteogenic potential of the polymer/hydrogel system. Our approach was to adopt native chemical ligation chemistries to assemble polyethylene glycol precursors that avail of both thioester and amide moieties to crosslink the polymers. This system provided two potential platforms to tether the biosilica phase through its surface modification with either amine (–NH2) or thiol (–SH) terminated end groups that will crosslink it into the polymer network. Multivalent PEG precursors were functionalised with N-hydroxysuccinimide (NHS) activated oxo-esters and N-cysteine (N-Cys) end groups, which in turn were tethered to the biosilica by either –NH2, or –SH terminated silanes. In bone, mechanical stability of the substitute material is important. Often the incorporation of an additional phase in hydrogels can disrupt the crosslinking network, causing excessive swelling and reduced strength. Our swell test data showed that the hydrogel, bioSi-PEG (NCL) crosslinked with –SH functionalised biosilica had the highest swelling rate, but remained less than 16% relative weight.

Biosilica functionalised with –NH2 showed a swelling profile similar to hydrogels only. Furthermore, improved mechanical properties were observed with the addition of biosilica (e.g. Young's modulus, 132.7kPA (hydrogels only); 189.7kPA (with Si) after 24hrs) which is important for mechanical load transfer when implanted in the skeleton. Gelation times in these types of surgeries are also important, as surgeons need to fix multiple fragments together and close the wound quickly. Our system was found to have a gelation time of <3 minutes when mixed at pH 7.4. To summarise, the first phase of the project tested three methods of incorporating biosilica into an adhesive hydrogel and showed that mechanical properties were improved, swell rates were within acceptable limits and gelation times were suitable for clinical applications. These were encouraging results and work progressed to the next phase.

The use of dietary Si and other naturally occurring amorphous silica have the potential to negate the resorption issues that are associated with synthetic silica. Its use has many advantages over its synthetic counterparts, however, issues such as purity, presence of endotoxins and cytotoxicity need to be addressed before it could be considered for medical applications therefore Phase 2 of the project investigated in vitro cytotoxicity of the biosilica in accordance with ISO standards 10993-05. Biosilica frustules (20um) were isolated from Cyclotella meneghiniana, sourced from the Mississippi river and grown as pure cultures. The organic phase of approximately 40% (measured by thermogravimetric analysis) was removed using a nitric acid digest. Biosilica frustules were then functionalised with amino and thiol groups and, along with unmodified frustules, were soaked in media for 24hrs. Both biosilica frustules and their soluble products released into the supernatant (known as conditioned medium) were used to assess cytotoxicity with a murine-monocyte macrophage (J774) cell line. Outcome measures were LDH release to measure damage to cell membrane, MTS to measure cell viability, a live-dead assay and release of pro-inflammatory cytokines (IL-6 and TNF). Native diatom frustules and those functionalised with amino groups showed no cytotoxicity or elevated cytokine release. Diatom frustules functionalised with thiol groups showed higher levels of cytotoxicity. Although these were still within acceptable limits, since Phase 1 tests had not demonstrated any significant difference between the amine or thiol groups, this result was used as the basis for choosing one functionalization method for progressing to in vivo testing (Phase 4 below).

Phase 3 tested cell response to the biosilica/hydrogel system developed in Phase one. In vitro tests using J774 were repeated to assess cytotoxicity and pro-inflammatory potential of the complete system, prior to testing the response of human mesenchymal stem cells (hMSCs), which were used as a model for bone cell response. The groups tested were hydrogels only, hydrogels loaded with functionalized biosilica (–NH2, and –SH terminated silanes) with end groups that crosslinked the network and biosilica without modification (i.e encapsulated into the gels uncrosslinked). With J774 cells, there was no cytotoxicity in any group. Furthermore, the compression test data, showed an increase in the modulus of hydrogels crosslinked with thiols with respect to time, which might suggest that whatever was leaching out of the biosilica frustules functionalised with thiol groups in the previous studies was now helping reinforce the hydrogel system. Cell viability results found that biosilica without modification induced the greatest cell response. Further work is required to identify the stimuli that evoked this response. For the hMSCs, cells were poorly attached to the hydrogels yet remained alive and attached on tissue culture plastic surrounding the hydrogels in the same well. There was no evidence of cytotoxicity and alkaline phosphatase activity levels suggested the cells were differentiating down the osteogenic lineage. Given the poor cell attachment, however, these experiments were not ideal to test cell response and a better protocol

is required for in vitro testing. Further work is planned in this area.

In Phase 4, native diatom frustules and those functionalised with amino groups were tested in a mouse cytotoxicity model, whereby the immunological response, organ toxicity (kidney, spleen, liver) and route of metabolism/excretion of silica were investigated. This was to ensure that when the Si frustules are not toxic if and when they are released from the degrading hydrogel when implanted in the body. Mice were injected with a single high dose injection of frustules or vehicle control and sacrificed at one day and 28 days post-injection. Histological results showed no organ toxicity in any of the groups relative to control. Si levels in blood and faeces showed that modified frustules are metabolised slower than native frustules, suggesting that physiochemical attributes influence their biodistribution and perhaps suggesting that modified frustules could retain their bioactivity for longer. This latter hypothesis will be tested by future in vivo testing in application specific animal models.

In summary, we have identified strategies for effectively incorporating biosilica frustules into our chosen hydrogel system; we have confirmed the non-toxic nature of the native biosilica and the modified frustules both alone, and when incorporated into the gels in in vivo and in vitro testing. The current hurdle, which this and other bone adhesive technologies need to overcome, is their ability to bond two surfaces with sufficient mechanical strength and the ability to withstand mechanical forces acting throughout the bulk of the material, while at the same time inducing bone formation in a complex physiological environment. Our current system needs better mechanical properties before it could be used as a standalone adhesive in hard tissue, however, the results to date have been promising and the potential of using biosilica as a model system to explore many unanswered questions on the role of silica in bone repair is a novel and exciting new direction for this research.

Powiązane dokumenty

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