Institution:	Imperial College London
Unit of Assessment:	12 Engineering
Title of case study:	Bioglass as an Osteogenic Synthetic Bone Graft and the Active Agent in Sensodyne Repair and Protect Toothpaste and Toothpaste Formulation Improvement
Period when the underpinning research was undertaken:	2000 - 2020

Details of staff conducting the underpinning research from the submitting unit

Name(s):	Role(s) (e.g. job title):	Period(s) employed:
Larry L Hench	Emeritus Professor of Ceramic Materials	1996 – 2005
Julian Jones	Professor of Biomaterials	2002 – present
Molly Stevens	Professor of Biomedical Materials and Regenerative Medicine	2001 – present

Period when the claimed impact occurred:	1 August 2013 – 31 December 2020
Is this case study continued from a case study submitted in 2014?	Yes

1. Summary of the impact

Bioglass based bone grafts have been used in more than two million patients; Imperial's work on cell response-enabled "**Osteostimulation**" enabling *NovaBone*® LLC revenue to grow to GBP16,000,000 in 2019, leading to its acquisition by Halma Group (UK) for GBP74,000,000 in 2020. In 2019, *Collagen Matrix* Inc. launched a new Bioglass containing composite graft (*OssiMend*) and applied for regulatory approval for "osteostimulation" in 2020.

Sensodyne Repair and Protect toothpaste contains Bioglass (NovaMin®) as the active ingredient. Bioglass particles provoke ***mineralisation*** of dentine. In 2017, GlaxoSmithKline (GSK) released a new formulation (known as "New Stronger Repair"), increasing sales of Sensodyne brand by 10% annually.

Our research has led to a new protocol being adopted by the International Commission on Glass (ICG) to determine the rate of mineralisation of glass, thus providing standard tests for industry using Bioglass in their products.

2. Underpinning research

I1 Osteostimulation

Cell Culture: Cell culture studies by Prof.s Larry Hench (Materials), Julia Polak (Medicine) and student Ioannis Xynos (2001), showed that dissolution products of Bioglass powder stimulate bone cells (human osteoblasts) to produce new bone [R1]. Gene expression increased due to dissolution products, e.g. insulin-like growth factor II (IGF-II), an abundant growth factor in bone, increased more than three-fold, without addition of other osteogenic supplements [R1]. Mineralised bone nodule formation also increased. Olga Tsigkou (PDRA), Professors Julian Jones and Molly Stevens later (in 2009) found a dose dependent effect, with the optimal concentration of soluble silica of 15-20 $\mu g mL^{-1}$ promoting highest metabolic activity [R2]. The result that the dissolution products of the glass stimulated bone cells explained why Bioglass outperformed other "bioactive" ceramics in terms of rate of bone repair.

In 2020, Jones, Dr Silvia Ferreira (PDRA) and Prof. Sara Rankin (NHLI, Imperial) carried out similar experiments with a collagen/synthetic hydroxyapatite/Bioglass composite (OssiMend® Bioactive, Collagen Matrix Inc, NJ) and found that its dissolution products stimulated bone cells more than those of the gold standard Bioglass at similar concentrations (again ~20 μgmL^{-1} of silica) [R3]. Gene expression studies and mineralisation assays showed that dissolution products of the new composite accelerated osteoblast differentiation, producing mineralised bone matrix more rapidly and moving quickly to the mature osteocyte phenotype. The enhanced mineralisation and gene expression were attributed to a synergistic effect of Bioglass dissolution and additional phosphate release from the composite [R3].

I2 Mineralising toothpaste formulations

The same Bioglass powder (NovaMin®) is the active ingredient in *Sensodyne Repair and Protect* (~5 µm particles at a concentration of 5% in paste). Fundamental research was carried out in the Materials Department at Imperial. Jones (PhD student at the time) and Hench reported how Bioglass particle size and dose [R4] affected pH rise, dissolution profiles and mineral formation (2001). Bioglass particles with particle sizes less than 5 µm were found to nucleate hydroxycarbonate apatite (HCA, the mineral component of enamel and dentine) within 22 hours in simulated body fluid when 2 $mgmL^{-1}$ or less was used, but when higher concentrations were used, calcium carbonate formed instead [R4]. This particle size was used in the formulation.

Later (2012-2020), Prof. Jones, with PhD students Esther Valliant and Anthony Maçon, investigated effect of variables in toothpaste formulation on HCA formation in artificial saliva: glass particle size, glass content, additional mineral content (2012-2015, [reports submitted to GSK]), and importantly fluoride source (2015). They also developed the testing method [R5]. Monofluorophosphate releases fluoride under enzyme action. Jones' team found that sodium fluoride improved mineralisation ability of the toothpaste, even in artificial saliva containing the enzyme alkaline phosphatase. Increasing the cleavage of monofluorophosphate reduced mineralisation, even though phosphate concentration in the solution increased, due to a pH buffering effect [R5].

I3 Standards

Variation in "bioactivity"/mineralisation testing methods led to Jones heading an international team to agree a unified protocol, which was ratified in round robin testing [R6]. He assembled the team, set the test conditions, based on previous work [R4] and collated the results from eight groups (each in a different country) [R6]. PhD students TB Kim and A Maçon ran the test with Jones at Imperial. Six commercial bioactive glasses were tested and compared to an ISO test. The unified method enabled the direct effect of the particle size on mineralisation to be determined (glass of a smaller particle size nucleated more HCA an earlier time point), which was not seen in the ISO test [R6].

3. References to the research

R1. Xynos, I.D., Edgar, A.J., Buttery, L.D.K., **Hench,** L.L., Polak, J.M. "Gene-expression profiling of human osteoblasts following treatment with the ionic products of Bioglass® 45S5 dissolution", *Journal of Biomedical Materials Research* 2001: 55: 151-157. DOI: 10.1002/1097-4636(200105)55:2<151::AID-JBM1001>3.0.CO;2-D.

R2. Tsigkou, O., **Jones, J.R.**, Polak, J.M., **Stevens, M.M.** "Differentiation of fetal osteoblasts and formation of mineralized bone nodules by 45S5 Bioglass® conditioned medium in

the absence of osteogenic supplements", *Biomaterials*, 2009: 30: 3542-3550. DOI: 10.1016/j.biomaterials.2009.03.019.

R3. Ferreira, S. A., Young, G., Jones, J. R., Rankin, S., "Bioglass/carbonate apatite/collagen composite scaffold dissolution products promote human osteoblast differentiation", Journal of Materials Science and Engineering C, 2021: 118: 111393. DOI: 10.1016/j.msec.2020.111393.

R4. **Jones J.R.**, Sepulveda, P., Hench L.L. "Dose-dependent behaviour of bioactive glass dissolution", *Journal of Biomedical Materials Research B*, 2001: 58B: 720-726. DOI: 10.1002/jbm.10053.

R5. Maçon, A.L.B., Valliant, E.M., Earl, J.S., **Jones, J.R.** "Bioactivity of toothpaste containing bioactive glass in remineralizing media: effect of fluoride release from the enzymatic cleavage of monofluorophosphate." Biomedical Glasses, 2015: 1: 41-50. DOI: 10.1515/bglass-2015-0005.

R6. Maçon, A.L. B., Kim, T.B., Valliant, E.M., Goetschius, K., Brow, R.K., Day, D.E., Hoppe, A., Boccaccini, A.R., Kim, I.Y., Ohtsuki, C., Kokubo, T., Osaka, A., Vallet-Regí, M., Arcos, D., Fraile, L., Salinas, A.J., Teixeira, A., Vueva, Y., Almeida, R.M., Miola, M., Vitale-Brovarone, C., Verné, E, Höland, W., **Jones, J.R.** "A unified in vitro evaluation for apatite-forming ability of bioactive glasses and their variants". Journal of Materials Science: Materials in Medicine, 2015:26:115 DOI: 10.1007/s10856-015-5403-9.

4. Details of the impact

I1. Osteostimulation: Underpinning research [R1, R2, R4] demonstrated that critical concentrations of Bioglass dissolution products stimulated bone cells to produce more bone matrix. This gave rise to the Food and Drug Administration (FDA) approved technique known as "Osteostimulation" where Bioglass (and other biomaterials) is introduced to harness the body's natural bone healing process. This technique and the term "Osteostimulation" has been adopted by NovaBone for their products (NovaBone LLC). It describes to surgeons why NovaBone® performs better than its competitors (gene stimulation), resulting in the company increasing its revenue by 40% to GBP16,000,000 at the end of 2019 [E1]. The impact of our research into Bioglass can be found in NovaBone's elevation to be the leading synthetic bone graft in the USA. This led to acquisition by Halma Group (Amersham UK) for GBP74,000,000 in 2020 [E1]. NovaBone has been implanted in more than two million patients. Earlier success led to new "osteostimulation" products such as a porous composite of collagen and Bioglass, NovaBone MacroFORM® in 2014. NovaBone is a company with an annual revenue of GBP16,000,000 and annual earning (adjusted EBIT) of GBP5,300,000 in 2019, and more than 30 employees [E1].

Due to the success of NovaBone, new osteostimulative Bioglass products have been released in Europe, e.g. Bonalive® putty (Bonalive Biomaterials Ltd, Finland, 2013, [E2]), which reaches over 40 markets, and GlassBONE™ putty (Noraker, France, 2017) [E10].

In 2019, in collaboration with Collagen Matrix Inc. (Oakland, NJ), our research helped to demonstrate that their new product, OssiMend® Bioactive Moldable Bone Graft Matrix, also exhibits osteostimulative properties similar to that of Bioglass. OssiMend Bioactive was approved by the FDA in 2019 [E3]. Additionally, Collagen Matrix, based on our research, has submitted a 510k application for the formal claim of the use of the term "Osteostimulation" to describe their product [E4, R3].

I2 Mineralising toothpaste formulations: The fact that dissolution products of Bioglass stimulated biomineralization [R1, R2, R4 and others] directly led to a toothpaste containing Bioglass particles (NovaMin®) to treat hypersensitivity. 57% of people suffer from

hypersensitivity (tooth pain) caused by fluid flow into exposed tubules in dentin that lead to nerve endings. When teeth are brushed with toothpaste containing NovaMin®, the particles dissolve and produce mineral with similar composition to enamel, which occludes the tubules. **GSK** acquired NovaMin® in 2009 for GBP135,000,000. *Sensodyne Repair and Protect* launched in 2011 and won Product of the Year in 2013 [E5]. Although this launch was outside the current REF period, the impact of Bioglass in toothpaste, continued since 2014. Our research on particle size and dose [R4], particularly in artificial saliva, led to selection of particle size, source and concentration in the paste [E6]. Combined with work on fluoride source [R5 and reports to GSK], the research led to improved formulation, including particle size and use of sodium fluoride as the fluoride source, launched as *Sensodyne Repair and Protect* ("New Stronger Repair") in 2017, in over 40 countries including most of the EU [E6]. It is now used in more than 2,300,000 households and is worth GBP15,700,000 of the UK sensitive toothpaste market with a 4% share of the total toothpaste market. The Sensodyne brand grew approximately 10% annually (2017- 2019) [E7a (p64), E7b (p48), E7c (p58)]. According to GSK, the brand is worth USD1,000,000,000 [E8]. Although it is impossible to apportion contributions of all factors that brought about this growth, the work in [R4, R5] and our research collaboration with GSK clearly played a significant part to the success of the *Sensodyne* range of toothpastes and more than GBP2,000,000,000 annual turnover of their Oral Health Division [E7a (p64), E7b (p48), E7c (p58)].

I3 Standards: Prior to 2015, medical device companies and research groups working on bioactive glasses all used a slightly different methods for testing bioactivity in vitro. Regulatory bodies such as the FDA and EU (CE marking) rely heavily on consensus standards, such as ISO and ASTM standards. Newly developed materials that are designed to be bioactive are often tested in simulated body fluids and compared to the original Bioglass. While an ISO test for investigating apatite formation on bioceramics exists, it does not hold for bioactive glasses. Under the auspices of the International Commission on Glass (ICG), Jones chaired the Technical Committee to establish a unified testing protocol to determine the rate of mineralisation of glass, which is based on the research reported in (R6) in 2015. The published protocol (R6) is used by Noraker and its CEO states: "*We use it as a comparative study to demonstrate that bioactive glasses form an HA layer in our biological risk assessment and also use it as a justification for not following the ISO 23317:2012 protocol.*" [E9]. The protocol now informs an ASTM (formerly known as American Society for Testing and Materials) working group (WK65452) to develop a standard for bioactivity [E10].

5. Sources to corroborate the impact

E1 https://otp.tools.investis.com/clients/uk/halma2/rns/regulatory-story.aspx?cid=140&newsid=1361067 Link archived here.

E2 https://www.medgadget.com/2013/11/bonalive-biomaterials-gets-ce-mark-for-bonalive-putty.html Link archived here.

E3 https://www.fdanews.com/articles/190979-collagen-matrixs-moldable-spinal-bone-graft-cleared-by-fda Link archived here.

E4 Letter from Director of R&D, Collagen Matrix.

E5 Sensodyne Repair and Protect wins Product of the Year 2013, which is voted for by consumers: http://www.talkingretail.com/products/product-news/sensodyne-announces-toothpaste-product-of-the-year-2013-win Link archived here.

E6 Letter from Innovation Research Lead, Oral Health R&D, GSK Consumer Healthcare, UK.

E7	Published Annual Reports confirming the value of the Sensodyne brand: a) GSK Annual Report 2017: https://www.gsk.com/media/4751/annual-report.pdf Link archived here. b) GSK Annual Report 2018: https://www.gsk.com/media/5349/annual-report-2018.pdf Link archived here. c) GSK Annual Report 2019: https://www.gsk.com/media/5894/annual-report.pdf Link archived here.
E8	GSK presentation "Sensodyne – One Billion Dollar Brand". PDF available here.
E9	Letter from CEO, Noraker stating the impact of Jones' research to their company.
E10	Letter from Spinode Consulting confirming the impact of our test protocol on the development of ASTM standard for bioactivity.