Chapter 2

Infection resistance of degradable versus non-degradable biomaterials: An assessment of the potential mechanisms

Reproduced with permission of Elsevier from Daghighi S, Sjollema J, Van der Mei HC, Busscher HJ, Rochford ETJ. Biomaterials, 2013; 34: 8013-8017
Abstract

Extended life expectancy and medical development has led to an increased reliance on biomaterial implants and devices to support or restore human anatomy and function. However, the presence of an implanted biomaterial results in an increased susceptibility to infection. Due to the severity of the potential outcomes of biomaterial-associated infection, different strategies have been employed to reduce the infection risk. Interestingly, degradable biological materials demonstrate increased resistance to bacterial infection compared to non-degradable synthetic biomaterials. Current knowledge about the specific mechanisms of how degradable biological materials are afforded increased resistance to infection is limited. Therefore, in this paper a number of hypotheses to explain the decreased infection risk associated with the use of degradable versus non-degradable biomaterials are evaluated and discussed with reference to the present state of knowledge.
Introduction

Increasing life expectancy has led to the use of different biomaterial implants and devices for the restoration and maintenance of human anatomy and function after trauma, surgery or general wear (1). However, there are several disadvantages associated with the use of implants, including the risks of limited healing, destructive inflammation and the development of biomaterial-associated infection (BAI) (2,3). Currently, commercial biomaterials are screened before use to minimise toxicity, inflammation and inappropriate immune reactions. However, the risk of infection associated with the use of biomaterial implants and devices is often overlooked during development, despite the fact that it is the primary cause of biomaterial implant and device failure. In addition, BAI usually shows little susceptibility to antibiotics making BAI cases difficult to treat, often requiring revision surgeries and implant removal (4).

Bacterial contamination of an implant can occur peri- and post-operatively and also many years later via haematogenous seeding from infections elsewhere in the body (1). Bacteria possess a wide range of adhesion molecules which target a vast array of surface chemistries and adsorbed proteins aiding their adhesion to a biomaterial surface or surrounding tissue yielding contamination of the surgical site (5,6). Upon adhesion, the bacterial phenotype changes, leading to the formation of a biofilm: an organized community of adhering bacteria embedded in a matrix of extracellular polymeric substances (EPS) (7). Bacteria in this mode of growth demonstrate an increased resistance to antimicrobials and effective removal by the immune system (8-10).

The presence of an implanted material alone increases the risk of infection dramatically, as first illustrated by Elek and Conen in 1957. It was demonstrated that $10^4$ times fewer bacteria were required to infect human volunteers receiving a suture compared to those without (11). This feature of decreased infection resistance has been attributed to the compromising of the immune system by the presence of “non-self” material which leads to the
development of a foreign body reaction. A foreign body reaction is a characteristic immune response to material in the body identified to be xenogenic and involves the recruitment of phagocytic cells (12). Depending on the material present, the foreign body reaction can lead to chronic inflammation, frustrated phagocytosis, granulation tissue development, formation of multinucleated foreign body giant cells, fibrous capsule development and the release of reactive oxygen species. It has been suggested that the host reaction to a foreign material may reduce the effectiveness of the immune system, generating a refractory period in which bacteria are not cleared effectively, leading to increased infection risk (12-15).

The consequences of a BAI are significant and include increased hospitalization times, treatment costs, the requirement for implant removal and tissue debridement, morbidity and in worst cases even death (16-19). Due to the severity of the potential outcomes of BAI, different strategies have been used in clinical applications to reduce infection risk. For example, chlorhexidine-releasing vascular catheters have been employed to reduce blood stream infections (20) and antibiotic-loaded bone cements and antibiotic-coated arthroplasties are applied in orthopaedic surgeries (21). The use of antimicrobials associated with implants may reduce infection risk, but moreover may stimulate the development of bacterial resistance. This is especially true, when these drugs are used and eluted for prolonged periods of time in concentrations below clinical efficacy. Therefore non-adhesive coatings (22), preventing initial bacterial adhesion, and coatings with contact-killing activity (23,24) are under development that may provide long-lasting functionality, without the drawbacks of current antimicrobial strategies.

Another clinically effective strategy to reduce infection risk is, where appropriate, to use degradable biomaterials. For example, hernia repair grafts composed of an a-cellular collagen scaffold from human cadaveric, porcine or bovine sources, show improved resistance to bacterial infection compared to non-biological grafts (25-27). The benefits of using degradable biological grafts to
decrease infection risk have been reported in a number of clinical and pre-clinical
in vivo studies (28-33). Animal studies comparing biological meshes with synthetic
meshes found biological materials to be more resistant to *Staphylococcus aureus*
(13,25,27). Also in clinical trials, biological materials have been shown to possess
higher bacterial clearance rates in patients with either a contaminated wound or a
history of infection. For instance, the results of a 5-year follow-up study suggested
that in infected or potentially contaminated fields where infection resistance of an
implant is required, placement of degradable, biological meshes is preferred over
non-degradable biomaterials (30). Therefore, degradable meshes of biological
origin are often recommended for the treatment of abdominal wall hernias in
high infection risk scenarios indicated by co-morbidity factors (smoking, Chronic
Obstructive Pulmonary Disease (COPD), obesity, an immune compromised state,
etc.) (34). Additionally, these degradable implants show a reduction in growth
restriction (35), pain (36), implant migration (37), requirement for secondary
revision and removal surgeries (38). However, the mechanisms by which
degradable materials are afforded this increased resistance to infection have yet
to be established. To date, a number of hypotheses have been proposed to
explain the reduced risk of infection of degradable versus non-degradable
biomaterials, these will be critically discussed in this article.

**Current hypotheses of infection resistance of degradable biomaterials**

*Increased vascularisation*

Increased vascularisation has been suggested to be a reason for the
higher infection resistance associated with the use of degradable materials
(39,40). Enhanced vascularisation may aid resistance by facilitating immune cell
infiltration into damaged and infected host tissue (41). The vascularisation
hypothesis is based on observations such as that made by Disa *et al.* that
degradable meshes cause increased angiogenesis and decreased infection risk
(42). It is unclear, however, whether there is a causal relationship between
neovascularisation and the so called “inherent” infection resistance of degradable grafts. To date this link can only be regarded as circumstantial and must be interpreted with great care, because of the complexity of immune response between implantation and outcome of healing and bacterial contamination. The processes of tissue healing, including neovascularisation, and host clearance of contaminating microorganisms are driven by the immune system, which in turn is affected by material choice (43,44). Neovascularisation is controlled by the production of cytokines and recruitment of cells to the site of healing. These same processes influence and are in turn influenced by other aspects of the immune response. For example, vascular endothelial growth factor is an important protein in the development of angiogenesis (45). However, this same protein is also chemo-tactic for macrophages and increases vascular permeability (46). Thus the presence of cytokines involved in promoting vascularisation may have direct implications on the host response to pathogens and this link may be more complicated than vascularisation alone influencing infection risk.

Reduction of the local immunological deficit

For many years, the “immunological deficit” associated with the presence of a foreign material has been linked to decreased infection resistance (11), though the nature of this feature has yet to be defined. The underlying principle is that the presence of a foreign material skews the immune responses away from the normal competency to remove pathogens. The responses to foreign materials include inflammation, necrosis, immune cell recruitment, differentiation and the release of numerous signalling molecules to cause an immune response relevant to the non-self material. However, to date, there is no consensus on which specific immune responses simultaneously promote tissue healing and effective pathogen removal. A clear point in case, is the cytokine IL-12 which promotes an inflammatory response by stimulating the differentiation of naïve T cells into TH1 cells. In the literature, both the presence of IL-12 releasing coatings and the
blocking of IL-12 by anti-IL-12p40 monoclonal antibodies have been shown to reduce BAI risk (47,48). Therefore, even on a single cytokine level there is controversy as to what is the desirable immune response. In addition to the specifics of immune responses, there are differing opinions as to how some of the broader outcomes of the immunological cascade affect infection risk. The lack of knowledge about the immune response to BAI and the outcome of the host response is a clear area for further research, both for degradable and non-degradable implant materials alike.

Traditional, non-degradable meshes have been shown to induce increased inflammatory interleukin (IL)-1, tumour necrosis factor (TNF) and immune cell recruiting chemokines and simultaneously decreased anti-inflammatory (IL-10 and IL-1 receptor antagonist) cytokine activity compared with treatment in the absence of an implant (49,50). Such immune responses to implanted non-degradable materials lead to a higher influx of inflammatory cells when compared with repair without an implant (49). Together, these features may prevent the immune response from being able to effectively target and clear bacteria. In contrast, the use of degradable materials may decrease the immunological deficit via two mechanisms. Firstly, degradable biomaterials are readily broken down and may not frustrate the immune system to the same extent as non-degradable materials, thus permitting immune responses to develop targeted to contaminating pathogens rather than to the material itself (51). There are a number of examples which support this hypothesis of lower immunogenicity. The use of degradable implants leads to a significant reduction in the recruitment of inflammatory cells when compared with non-degradable implants (30,52,53). In addition, degradable materials have been shown to avoid the formation of multinucleated foreign body giant cells, a key sign of a frustrated immune reaction and the foreign body reaction, when compared with non-degradable equivalents (40). Furthermore, xenogenic degradable meshes have been shown to stimulate the release of IL-10, an anti-inflammatory, suppressive cytokine, whilst
non-degradable prolene stimulated inflammatory signals such as TNF-α and interferon gamma (IFN-γ) in a murine model (54). These features suggest that degradable meshes have a lower immunogenicity than their non-degradable equivalents which may subsequently permit a specific anti-bacterial immune response to develop contributing to a decreased infection risk. Whether this decreased immunogenicity is due to the degradable nature, the biological origin of the mesh materials or a combination of both has yet to be clarified.

In addition to lowering immunogenicity, the full degradation of an implant material may also restore the immune system to full efficacy. In a study by Daghighi et al., it was observed that amongst degradable materials, the degree of infection correlated to the extent of degradation in vivo (13). Over a 28 day study, infection persisted in animals with non-degradable or incompletely degraded implants; whilst in contrast, infection was no longer present in animals after the material had completely degraded. The persistence of infection around degraded materials until complete degradation suggests that the presence of any amount of biomaterial, regardless of type, may prevent the immune response from effectively eradicating the infection. This supports the hypothesis that the elimination of foreign material is an effective method to prevent infection.

Analogous to the prevention of infection associated with the complete degradation of implants, success of therapies in case of infected non-degradable materials seems to only be achieved by revision surgery and implant removal. For instance, several clinical studies (18,19) have shown that antibiotic therapy is unsuccessful before foreign body removal, e.g. in case of coronary stent infections (55) and catheter-related urinary tract infections (18). According to a study of coronary stent infections, only early sub-acute infections (occurring less than 10 days after implantation) were amenable to antibiotics therapy, while in the cases of late infections (occurring more than 10 days after implantation) a surgical intervention was necessary to relieve sub-chronic symptoms, combining foreign body removal and antibiotic therapy (55,56). These cases illustrate that antibiotics
alone are often ineffective to fully resolve the biomaterial associated infection and fail to treat bacteria in a biofilm mode of growth. The removal of an implant by revision surgery or degradation may not be only associated with the elimination of the surface associated biofilm, but also may restore the immune system and re-activate effective phagocytosis to clear surrounding tissue from infecting microorganism. Both aiding in the removal of pathogens and preventing future infections from developing.

**Influence of degradation on bacterial adhesion**

Further to increased vascularisation and the restoration of the immune function, the degradation of implants directly reduces the area available for bacterial colonization and subsequent biofilm formation. Initial bacterial adhesion to an implanted material is believed to be a key feature in the direct effect of degradation on development of BAI. Following colonisation of a surface, bacteria can form a resistant biofilm which propagates infection into the surrounding tissue. When in this biofilm mode of growth, bacteria are afforded increased protection from the host immune responses by being encapsulated in EPS (57). An example of this is the ability of EPS to alter complement activation (58,59). Complement activation is a fundamental part of the early innate immune response and has multiple functions (60). The first action is the binding of complement factor C3b to the foreign material which opsonises the surface allowing more efficient phagocytosis (61). Additionally, the release of C3a and subsequent cascade of complement factors causes the recruitment of immune cells to the site of activation and inflammation (62-64). Biofilm EPS shields the resident bacteria from direct opsonisation and also causes non-specific complement activation, thus preventing a targeted immune response (58). Furthermore, the biofilm mode of growth has been shown to prevent effective phagocytosis by immune cells (65-67). This is due to the EPS buffering the bacteria from the phagocytes and also by presenting a much larger object to engulf, which
can lead to frustrated phagocytosis (68). Frustrated phagocytosis causes the non-specific release of reactive oxygen species, defensins and lysozyme from phagocytes and depletes the capability of these cells to react to specific threats, therefore decreasing infection resistance. Additionally, the EPS of a biofilm protects the resident bacteria by restricting the diffusion of antimicrobials including those released from phagocytes, further decreasing the ability of the immune response to remove infecting bacteria (69). This cascade of events that commence after initial bacterial adhesion to a biomaterial implant or device surface may not occur when bacterial adhesion proceeds on a degradable material. Particularly surface eroding degradable materials pose an unstable receding biomaterial surface from which adhering bacteria are continuously shed into the body. In this respect it would be of interest to determine whether rapidly degrading materials prevent the formation of biofilm more readily than a slowly degrading material.

A relationship between the rate of degradation and the risk of developing BAI, i.e. the use of degradable materials over non-degradable materials in general, is indicated by the eradication of infection achieved by revision surgery and implant removal. Several clinical studies (18,19) have shown that antibiotic therapy is unsuccessful before foreign body removal, e.g. in case of coronary stent infections (55) and catheter-related urinary tract infections (18). According to a study of coronary stent infections, only early sub-acute infections (occurring less than 10 days after implantation) were amenable to antibiotics therapy, while in the cases of late infections (occurring more than 10 days after implantation) a surgical intervention was necessary to relieve sub-chronic symptoms, combining foreign body removal and antibiotic therapy (55,56). These cases illustrate that antibiotics alone are often ineffective to fully resolve BAI and fail to treat bacteria in a biofilm mode of growth. The removal of an implant by revision surgery or degradation may not be only associated with the elimination of the surface associated biofilm, but also may restore the immune system and re-activate
effective phagocytosis to clear surrounding tissue from infecting microorganism. Both aiding in the removal of pathogens and preventing future infections from developing.

**Naturally occurring anti-bacterial peptides**

A final hypothesis to why degradable, biological materials used in soft tissue repair may resist infection is due to naturally occurring anti-bacterial peptides within the material (70). Products isolated after the degradation of porcine extracellular matrices have been shown to possess antimicrobial activities against both *S. aureus* and *Escherichia coli* (70,71), whilst the intact matrices did not (72). The exact nature of these released antimicrobials has yet to be determined and differs in activity between sources of the material (71). The release of these compounds may contribute to the infection resistance of these biological degradable implants.

**Summary and perspectives**

A number of hypotheses have been presented here, suggesting different proposed mechanisms for the improved infection resistance of degradable versus non-degradable materials, as schematically summarized in Fig. 1. Likely, no single mechanism will be responsible for the increased infection resistance afforded by the use of degradable materials but a combination of different ones involving the material itself, the way in which bacteria interact with the implant and perhaps most importantly the host responses to both the material and bacterial contamination. The inherent infection resistance of degradable biological materials may profoundly impact the design and use of a wide range of implants in the future including surgical meshes, drug delivery technologies and bio-scaffolds in tissue engineering and regenerative medicine. To identify further specific mechanisms of how degradable materials influence infection risk, the full
immune response towards degradable materials in the absence and presence of infection should be investigated. Additionally, the ability of bacteria to form biofilms on these materials \textit{in vivo} should be characterized. Ultimately, the knowledge gained about these degradable materials may translate into additional therapies e.g. degradable coatings based on currently non-degradable implants and immune-modulatory permanent implants which mimic the effect of degradable meshes on the host immune responses to promote infection resistance.
Figure 1. Schematic summary of possible mechanisms responsible for an increased infection resistance of biodegradable versus non-biodegradable materials, as operative at the time of implantation and after bacterial adhesion and/or biofilm formation on the material over time.
Chapter 2

Infection resistance of degradable biomaterials

References

30. Franklin ME, Jr., Trevino JM, Portillo G, Vela I, Glass JL, Gonzalez JJ. The use of porcine small intestinal submucosa as a prosthetic material for
Chapter 2  Infection resistance of degradable biomaterials


58. Kristian SA, Birkenstock TA, Sauder U, Mack D, Gotz F, Landmann R. Biofilm formation induces C3a release and protects Staphylococcus
epidermidis from IgG and complement deposition and from neutrophil-
al. Complement activation by Pseudomonas aeruginosa biofilms. Microb 
60. Trouw LA, Daha MR. Role of complement in innate immunity and host 
61. Lambris JD, Ricklin D, Geisbrecht BV. Complement evasion by human 
C3a and C5a stimulate chemotaxis of human mast cells. Blood 
1997;89:2863-70.
63. Dunkelberger JR, Song WC. Complement and its role in innate and 
64. Ward PA, Cochrane CG, Mueller-Eberhard HJ. The role of serum 
1965;122:327-46.
65. Leid JG, Willson CJ, Shirliff ME, Hassett DJ, Parsek MR, Jeffers AK. The 
exopolysaccharide alginate protects Pseudomonas aeruginosa biofilm 
bacteria from IFN-gamma-mediated macrophage killing. J Immunol 
66. Meluleni GJ, Grout M, Evans DJ, Pier GB. Mucoid Pseudomonas 
aeruginosa growing in a biofilm in vitro are killed by opsonic antibodies to 
the mucoid exopolysaccharide capsule but not by antibodies produced 
during chronic lung infection in cystic fibrosis patients. J Immunol 
Staphylococcus aureus biofilms prevent macrophage phagocytosis and 
al. Polysaccharide intercellular adhesin (PIA) protects Staphylococcus 
epidermidis against major components of the human innate immune 
70. Sarikaya A, Record R, Wu CC, Tullius B, Badyak S, Ladisch M. Antimicrobial 
Antibacterial activity within degradation products of biological scaffolds 
72. Holtom PD, Shinar Z, Benna J, Patzakis MJ. Porcine small intestine 
submucosa does not show antimicrobial properties. Clin Orthop Relat Res 